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Effect of Sub-Clinical Parasitism on the Absorption of Nutrients from the Gastro-Intestinal Tract of Sheep with Special Reference to Calcium and Phosphorus

by

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ABSTRACT

Recent studies have established that abomasal and intestinal parasitic infections can reduce the growth and mineralisation of the skeleton of growing lambs. One possible cause for the skeletal effect is that the parasites induce a mineral deficiency by reducing the absorption of dietary Ca and P and/or increasing the endogenous loss of these two elements. The importance of these factors was investigated in this study.

This was achieved by using growing lambs fitted with cannulae in the rumen, abomasum and ileum and infected daily with Trichostrongylus colubriformis larvae, Ostertagia circumcincta larvae or no larvae. The lambs were fed the complete diet Ruminant A and the flow rates were measured by reference to soluble and particulate radioactive markers. The endogenous fraction of Ca and P in faeces and digesta was measured by the method of isotope dilution involving a single intravenous dose of ^{32}P and ^{45}Ca.

A preliminary investigation showed that the best method for calculating the fraction of Ca and P which is of endogenous origin was the area method using a two day delay and that the SR in plasma was more reliable than the SR in urine.

O. circumcincta infection was without effect on the absorption and secretion of Ca and P in the digestive tract, other

than producing a small but significant increase in the endogenous faecal Ca excretion. There was, however, a striking increase in the flow of digesta at the abomasum, the DM and Na content of which was consistent with an increased secretion from the mucus - type cells in the abomasal mucosa. The extra digesta was absorbed in the small intestine.

T. colubriformis infections reduced the absorption of exogenous P by about 30% and increased losses of endogenous P due to the action of worms on the mucosa of the small intestine. The net effect of this was to induce a P deficiency in TC lambs leading to a reduced flow of salivary P and a reduction in the plasma concentrations of P. The effect of T. colubriformis on Ca metabolism was limited to an increase in endogenous faecal excretion.

Effect of sub-clinical parasitism on the absorption of nutrients from the gastro-intestinal tract of sheep with special reference to calcium and phosphorus

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Sheep flocks at pasture are invariably infected with worms. In many countries, particularly in the tropics and sub-tropics where conditions are often more favourable for the development and survival of infective stages, gastro-intestinal parasites are recognised as a major cause of death. Even more significant on a global basis are the losses resulting from inferior weight gains, wool growth, condemnation of carcases and organs, as well as the cost of drugs and veterinary care. The economic impact of these effects is impossible to quantify, but in Europe alone where strategic control measures are often incorporated into farm management systems, the damage is over a hundred million pounds annually (Urquhart and Armour, 1973). Parasites therefore pose serious problems to the livestock industry.

Efficient conversion of herbage to animal products by sheep depends upon effective control of parasitism of which the helminth diseases of the gastro-intestinal tract and liver are a major component. The clinical manifestations of such diseases are readily recognised. Sub-clinical disease states in which sheep appear normal but are performing below their full potential are more difficult to recognise until there has been considerable loss of production, and require nutritional techniques to demonstrate their precise

significance.

The problem is that of differentiating between nutritional constraints, such as food intake and quality, and parasitism when both are expressed in the same attributes - body weight gain, milk or wool production or efficiency of food conversion.

Estimation of food intake and quality in grazing animals requires sophisticated nutritional techniques unsuitable for commercial farms. It is only by comparisons with worm-free sheep which demonstrate the nutritional or management limitations on production that the true effects of sub-clinical parasitism can be demonstrated.

There is no direct evidence from the field on the extent of the adverse effect of sub-clinical worm infections on animal production due mainly to the difficulty in producing worm-free sheep under field conditions. Most of the relevant information comes from feeding trials with animals given known numbers of infective larvae.

In order to allow nutritional studies to be made, a relatively uniform and stable response to parasitism is required. This can be achieved by small but continuous larval doses, a pattern of infection which many sheep experience in the field (Sykes and Coop, 1976a). Earlier workers (Kates et al, 1959; Armour et al, 1966; Roseby, 1973; Reveron et al, 1974b) have used single doses of larvae which are not comparable with the continuous daily ingestion of small numbers of infective larvae by the

majority of lambs at pasture.

Furthermore, it is difficult to judge the size of challenge to use because of individual variation in resistance to parasitism, some animals develop clinical signs, inappetance leading to death, whereas others throw off the infection and are recovering during the time of experimentation. This difference in technique in stabilising infections may be responsible for the many conflicting results as to the way parasitism affects the host and must be taken into account when assessing evidence.

The most important parasites of sheep in Great Britain are Ostertagia spp and Trichostrongylus spp. Reid and Armour (1975) confirmed previous findings (Morgan et al, 1951; Wilson et al, 1953; Parnell et al, 1954) that nematode worm burdens in hill ewes vary on a seasonal basis. Reid and Armour (1975) found the major nematode genus present in the alimentary tract was Ostertagia, with O. circumcincta the predominant species. An absolute increase in total nematode burden and faecal egg count was apparent in the ewes commencing in late pregnancy, reaching a maximum during lactation and falling again in autumn and early winter. The worm burdens of the lambs were always low in autumn and early winter with Ostertagia spp being the major genus present during the autumn and Trichostrongylus spp being the predominant genus during the early winter.

ABOMASAL PARASITISM

The most important abomasal parasites are Ostertagia circumcincta, Trichostrongylus axei and Haemonchus contortus.

Grazing sheep ingest the infective third stage larvae which exsheath and penetrate the abomasal glands. The fourth stage larvae develop in the glands and by about day eight emerge onto the surface of the mucosa and continue their development to adult worms (Armour et al, 1966). Adult female parasites start to produce eggs about three weeks after initial infection.

The invasion of host tissues by parasites elicits a vast array of reactions, many of which can be attributed directly to penetration migration, and the feeding habits of the parasite concerned. For example, the essential lesion associated with O. circumcincta and T. axei is the destruction of gastric glands coinciding with the growth of developing larvae within, and their subsequent emergence from, the glands onto the mucosal surface (Armour et al, 1966; Soulsby, 1968). In the case of H. contortus, larval development is usually within or below the mucosa and most damage is caused by the adult worm which mutilates the mucosa with its piercing mouthparts and thereby produces intense haemorrhage.

The extensive replacement of functional cell types by undifferentiated ones, and the epithelial hyperplasia and inflammation responsible for the thickening of almost all parasitised mucosal surfaces have to be added to the above

characteristics. Such proliferative changes result in functionally immature cells being replaced in secretory and absorptive areas, and these changes promote the separation of inter-cellular junctional complexes (the so-called "leak lesion"), and thereby produce mucosae with enhanced permeability to macromolecules (Murray et al, 1970).

Coop et al (1977) found that hypertrophy of the fundic mucosa and presence of lesions specifically associated with the penetration of worm larvae, were the main features in sheep infected with O. circumcincta.

EFFECT OF DIFFERENT LEVELS OF INTAKE OF O. CIRCUMCINCTA ON THE OUTPUT OF EGGS IN LAMBS

In the UK, when lambs begin to graze they acquire worm infection as a result of residual infection surviving on the herbage from the previous year's grazing. An increase in pasture larval infection occurs in June and July. These larvae are derived from two sources; eggs passed by the lambs as a result of initial infections, and from an increased faecal egg output by their dams during the post-parturient period. A third, and often massive, larval population occurs during August and September derived from eggs passed by the lambs as a result of worm burdens acquired during the second wave of pasture infection.

It has been recognised for a number of years that an increase in the faecal nematode egg count of the ewe occurs in the Spring (Taylor, 1935), the so-called 'spring rise' (Morgan and Sloan, 1947; Cushnie and White, 1948; Naerland,

1949; Morgan et al, 1950; 1951; Crofton, 1954; Spedding and Brown, 1956; Field et al, 1960; Connan, 1968).

Gibson and Everett (1976) showed, using different levels of intake of O. circumcincta larvae, that a group of lambs, which experienced a low initial (residual) infection and a moderate second (spring rise) infection did better than a group of lambs which received only the spring rise infection. The former group showed little change in weight gain and produced a low egg output which would result in a low residual infection the following year. The latter group showed a poor weight gain and produced a high faecal egg output, which in the field would result in a high residual infection. Therefore, if the lambs experience a light residual infection, better results will be obtained than if they graze clean pasture, if no steps are taken to control the spring rise.

Gibson and Everett (1977) showed that infections which produced a high degree of resistance to subsequent infection, were responsible for a significant output of worm eggs and under field conditions this residual infection would be damaging to susceptible lambs which subsequently grazed the pasture. Therefore, after lambs have experienced an infection to stimulate the development of resistance, care must be taken to avoid exposure of susceptible young stock to any high levels of pasture larval infection which may result.

Faecal egg counts are recognised as an insensitive quantitative measure of pathogenicity of parasitic

infections. Coop et al (1977) found using different levels of O. circumcincta intake that the faecal egg counts were similar in all groups thus confirming the findings of Michel (1969a; 1969b) using O. ostertagi in calves that the total egg output of the whole population was restricted to a limit and was the same whether a few or many worms were present.

EFFECTS ON FEED INTAKE, BODY WEIGHT AND BLOOD COMPOSITION

Feed intake and Body weight

The importance of reduced feed intake has been examined by offering to uninfected animals the amount of food consumed daily by their infected partners (pair-feeding).

Single large infections in sheep and cattle (Jennings et al, 1966; Holmes and MacLean, 1971) have shown that appetite was depressed during emergence of fourth stage larvae from the gastric glands, but that it subsequently recovered. With continuous larval intake of O. circumcincta (Coop et al, 1977), food dry matter (DM) intake and DM digestibility were rapidly reduced and remained so throughout the period of infection, probably reflecting damage from continuous development of larvae. Coop et al (1977) reported that parasitism of the abomasum caused a 22% reduction in food intake. This persistent depression of appetite may be of considerable significance in field infections since it suggests that intermittent anthelminthic treatment may not remove the appetite depressant effect when larval intake is continuous.

The reduced feed intake is an important factor in the

reduction of growth rate. However Coop et al (1977) showed that the reduction in growth rate was due not only to depression of appetite but also to reduction in efficiency of food utilisation since infected sheep grew at only half the rate of pair-fed controls.

Gibson and Everett (1976) observed that animals experiencing infection for the first time showed a sharp loss of weight when the infection became established, but groups given a second or third infection either showed no effect or much less severe effects on re-infection.

Blood composition

Pepsinogen Increased plasma pepsinogen concentrations have been found in cattle infected with high doses of O. ostertagi larvae (Jennings et al, 1966; Anderson et al, 1966) or T. axei larvae (Ross et al, 1967; 1968). Similar findings were recorded in sheep dosed with O. circumcincta larvae (Armour et al, 1966; Coop et al, 1977).

Elevated serum pepsinogen concentrations have been found in association with natural outbreaks of Type II ovine ostertagiasis (Reid and Armour, 1973) and Reid and Armour (1975) considered serum pepsinogen concentration of some use in assessing abomasal damage in grazing sheep.

Proteins Hypoalbuminaemia occurs in many gastro-intestinal helminthiases and is a common feature of clinical ostertagiasis (Anderson et al, 1965; Holmes and MacLean, 1971). However, Coop et al (1977) found that hypoalbuminaemia was never severe even when performance was markedly affected.

They also reported that there was no significant differences in total serum protein, globulin or urea N concentrations between infected and control groups.

Calcium and Phosphorus In two experiments carried out by Waymack and Torbert (1969b), using calves dosed with Ostertagia spp., there was inconsistency in patterns of plasma calcium (Ca) concentrations. In one experiment (65,000 larvae administered) plasma Ca decreased immediately after dosing and continued to decrease until 28 days postinoculation. A sharp decrease occurred between the 21st and 28th day after dosing, but this was followed by an increase until the 35th day after dosing. In the other experiment (45,000 larvae administered) there was a marked increase in the level of plasma Ca near the 21st day postinoculation. After this increase, the concentration decreased almost as rapidly as it had increased. This was then followed by another increase near day 42 and a subsequent decrease. These workers also reported very little variation in plasma magnesium concentration.

Waymack and Torbert (1969a), using calves dosed with Ostertagia spp., observed plasma levels of bound and free phosphorus (P) over a 30 day infection period. Free plasma P decreased until the 12th day, after which an increase continued to the end of the 30 day period. The plasma-bound P reached a peak near the 21st day and then decreased till the end of the 30 day period. Patterns of total P concentration in the plasma were also reported. Total plasma P level was relatively unchanged for the first

10 days after dosing. By the 12th day, an increase in plasma P was evident, and a significant increase continued until the 23rd day. Afterwards, the plasma level decreased until the end of the experiment.

In two experiments, undertaken by Coop et al (1977), using lambs dosed with O. circumcincta, the changes in the concentration of Ca and P were conflicting. In the first experiment, the Ca concentration was unaffected but serum P concentration decreased in the animals dosed with 5,000 larvae/day. Conversely, in the second experiment, serum P concentration was unaffected, but serum Ca concentration, in animals dosed with 4,000 larvae/day, decreased.

EFFECTS ON FOOD UTILISATION

Sykes and Coop (1977), using lambs dosed with O. circumcincta, observed that the apparent digestibility of nitrogen (N) was severely reduced in the infected sheep two to three weeks after the commencement of infection, but this effect gradually became less marked with time. In the pair-fed sheep apparent digestibility of N increased with time. There were no significant differences between groups in urinary excretion of N. The infected sheep had lower balances of N than the pair-fed sheep, the difference being greatest two to three weeks after commencement of dosing when the infected sheep were in negative N balance.

Horak and Clark (1964) found that apparent digestibility of N decreased after a single dose of 300,000 larvae, but the interpretation of their data was complicated by virtually

complete anorexia. Due to large variation not only between infected animals but also between consecutive weeks in control animals, Parkins et al (1973) were not able to assess the significance of a reduction in mean digestibility one to two weeks after a single infection with 1,000,000 O. circumcincta larvae.

A reduction in N digestibility is probably due to reduced protein digestion, which in turn could result from the loss of differentiation and function of acid-secreting parietal cells and pepsinogen - secreting chief cells which occurs after infection of sheep with O. circumcincta (Armour et al, 1966; McLeay et al, 1973; Coop et al, 1977).

Digestibility of N returned almost to normal during the course of infection despite abomasal pH values of up to 6.0 when the animals were slaughtered (Sykes and Coop, 1977). Sykes and Coop (1977) suggested that the reduction in proteolytic digestion in the abomasum may have been followed by a gradual compensation by pancreatic proteolytic digestion.

In addition to the reduction in appetite and the small reduction in energy digestibility Sykes and Coop (1977) observed that a major factor in reducing animal performance was a drastic reduction in the efficiency of utilisation of metabolisable energy shown mainly in reduced fat deposition. It is not yet clear whether this is due to changes in metabolic rate or in the efficiency of utilisation of absorbed nutrients for maintenance or growth.

The above workers reported that the mean Ca and P balances of the groups showed a trend for increasing balances with time and that there was a greater mineral balance in the pair-fed controls than in the infected sheep, the latter being the most marked for Ca. Two to three weeks after infection the infected sheep were in negative mineral balance. The relative contributions of dietary and endogenous mineral components of the faeces were not determined.

EFFECTS ON THE SKELETON

Chronic abomasal parasitism reduces bone size and causes osteoporosis. This is significant for animal production since skeletal size in part determines the capacity of the young growing animal to accumulate muscle.

Sykes and Coop (1977) observed that the bone mineral deposition (Ca and P) was only 35% of that in unaffected sheep. This was confirmed by chemical pathology of individual bones which demonstrated comparable reductions in bone ash deposition (Sykes et al, 1977). Induced mineral deficiency could not be diagnosed on blood chemistry or on histological and chemical examinations of bones and these workers concluded that other factors which influence growth of bone matrix such as energy or protein availability or even specific hormonal changes induced in the host may be important. These latter aspects of parasitism are poorly understood, although a reduction in the plasma concentration of thyroxine does occur in chronic parasitism (A R Sykes and R L Coop, personal communication).

EFFECTS ON ABOMASAL FUNCTION AND GASTRIC SECRETION

The abomasum has exocrine secretory, endocrine and motor functions similar to those of the body and antral regions of the simpler stomach of species such as the cat, dog and man. Secretory activity of the abomasum appears to be regulated by a complex interaction of neural and hormonal stimuli similar to those operating in the stomach in other mammalian species (Makhlouf, 1974). Parietal cells are stimulated to secrete HCl, chief cells pepsinogen and the G cells (endocrine cells of the antrum) gastrin, by the vagus nerve.

When parasites of the genus Ostertagia become established in the abomasum there are marked changes in abomasal function. A major result of the altered abomasal secretion is a failure to maintain the acidity of the abomasal contents.

Studies have been carried out using sheep infected with O. circumcincta and fitted with separated fundic pouches and abomasal cannulae placed in the infected abomasum and the non-infected pouch.

McLeay et al (1973) reported that after a single infection the first changes in abomasal function occurred by the fourth day when increases in plasma pepsinogen concentrations and sodium levels of abomasal contents were detected. Holmes and MacLean (1971) postulated these changes may be the result of a permeability reabsorptive lesion.

After infection with O. circumcincta, it has been shown

that there are contrary reactions of the abomasum and the pouch. Whereas the main part of the abomasum shows a loss of the capacity to maintain the acidity of its contents, which may rise to, or above, pH 7.0 twelve or more days after infection, the pouches secrete more acid and at a higher concentration (McLeay et al, 1973; Anderson et al, 1976).

These changes are accompanied by a profound hypergastrinaemia and they are abolished by antrectomy. Since increased secretion and hypergastrinaemia are evident prior to any change in pH, it would appear that although both are maintained by the high pH, they are probably initiated by mechanical and/or chemical stimulation of the antral mucosa by parasites which happen to lodge in that general area. Whether this acts indirectly on ganglion cells or directly on the gastrin secretory cells remains to be established (Titchen and Anderson, 1977).

INTESTINAL PARASITISM

Two important small intestinal parasites are Trichostrongylus colubriformis and Trichostrongylus vitrinus.

Infections of the small intestine are characterised by mucosal and villous atrophy or flattening, and sparse stunted microvilli. Barker (1975) reported that three days after a single infection with 80,000 T. colubriformis larvae some shortening of the villi and elongation of the crypts was evident, eight days after infection the villi were short, 12 days after infection subtotal to severe

villous atrophy with leaks and erosions in the epithelium were seen and 16 days after infection severe villous atrophy with extensive erosions was evident. At all times after infection populations were densest in the first, second or third metre of intestine, and there was no evidence of population shifts with time.

Coop and Angus (1975) observed that continuous exposure to T. colubriformis larvae produced an atrophic mucosa and a deficiency of brush border enzyme activity in the anterior small intestine. There was extensive flattening of the mucosa. They postulated that the reduction in enzyme activity may result from either a decrease in the total number of functional epithelial cells on the villi or from a failure of maturation of surface epithelial cells. Symons et al (1971) reported that in nippostrongylosis of rats there was replacement of mature epithelial cells by immature crypt-like cells.

Coop et al (1976) found that extensive villous atrophy, flattening of the mucosa and intense infiltration by round cells and eosinophils were the main features in sheep infected with T. colubriformis. They also found that parasites were numerous in affected areas, were usually embedded in channels in the superficial mucosa, and that the intestinal crypts were elongated and contained numerous mucus-secreting cells and mitotic figures.

EFFECTS ON FEED INTAKE, BODYWEIGHT AND BLOOD COMPOSITION

Feed intake and Bodyweight

Chronic infection with T. colubriformis in growing lambs with no previous worm experience causes a gradual reduction in food intake from the time that adult worms become established, and, there are similar effects on weight gain. With continuous larval intake Sykes and Coop (1976b) found that over the whole experiment the reduction in food intake was 9% but in the last few weeks it was 16-20%.

Variable responses have been recorded as a result of single acute infection. Reveron et al (1974b) using a single dose of 40,000 larvae, showed an 18% and 24% reduction in appetite in 'slightly' and 'severely' affected animals respectively, and this reduction was maintained over an 11 week period, but Roseby (1973) using a dose of 30,000 larvae found a rapid 30% reduction during the third week of infection but with subsequent recovery of appetite.

The major factors responsible for poor growth rate in infected animals are the reduction in food intake and, as in abomasal parasitism, the reduced efficiency of use of digested energy. Coop et al (1976) reported that the uninfected groups consumed 8.6 (control group) and 9.8 (pair-fed control group) kg DM per kg of body weight gain whereas the infected group consumed 15.5 kg. Andrews et al (1944) similarly found that lambs chronically infected with T. colubriformis consumed almost twice as much food as uninfected controls per kg gain in bodyweight.

Blood composition

Proteins Coop et al (1976), using lambs dosed with

T. colubriformis, reported that hypoalbuminaemia developed in the infected animals three weeks after infection and hyperglobulinaemia was present from week six. They also reported that there was no significant difference in plasma total protein or urea concentration between the groups at any stage. Hypoalbuminaemia in trichostrongylosis is thought to result from protein leakage into the gastro-intestinal tract (Barker, 1973) although an osmoregulatory response to the hyperglobulinaemia may also be involved.

Calcium and Phosphorus Coop et al (1976) reported that plasma P concentration rapidly decreased during the third week of infection in the infected group, remained lower than in the pair-fed control group for nine weeks and recovered slightly towards the end of the experiment.

They noted that the decline in plasma P concentration three weeks after infection coincided with the establishment of adult worms in the small intestine. Hypophosphataemia was associated with normal plasma Ca levels, which agrees with the findings of Horak et al (1968) and Reveron et al (1974a) for acute T. colubriformis infection. Field et al (1975) suggested that this pattern of hypophosphataemia and normocalcaemia only occurs in a double deficiency of Ca and P, and it seems probable that intestinal parasitism impaired both Ca and P absorption. This is consistent with the evidence that Ca and P absorption occurs in the small intestine (Smith, 1969; Pfeffer et al, 1970).

EFFECTS ON FOOD UTILISATION

Sykes and Coop (1976b), using lambs dosed with T. colubriformis, observed that there was no significant difference between the pair-fed control + infected groups in the digestibility of energy or N during weeks six to seven and 12 to 13 after infection. Although intakes of apparently digested N were similar, N retention in the infected group was lower during week six to seven as a result of an increased urinary N excretion. During week 12 to 13 the N balances were reversed because urinary N excretion was lower in the infected group, but the difference in retention between groups was not significant.

The lack of effect of parasitism on the digestibility of the gross dietary energy and nitrogenous constituents supports the conclusions of Andrews et al (1944), Roseby (1973) and Reveron et al (1974b) regarding the effects of the parasite. Evidence for a reduction in the digestibility of N due to chronic infection with T. colubriformis was obtained by Franklin et al (1946) and Barger (1973), although, in the former, the effect seemed rather erratic and did not appear in all the balance trials.

Sykes and Coop (1976b) showed that protein deposition was impaired by parasitism. Increased liver protein synthesis has been demonstrated in acute infections (Symons et al, 1974) but the extent to which these changes can be simply attributed to increased plasma protein leakage into the gut and therefore a compensatory increase in albumin synthesis in the liver is not clear. Serum proteins

constitute only one fraction of the endogenous losses into the digestive tract, which include digestive secretion, mucus and sloughed epithelial cells.

Endogenous protein secretions are considered to be efficiently reabsorbed as amino acids in distal regions of the tract but increased protein recycling as a result of parasitic damage to the gastro-intestinal mucosa must impair overall efficiency of amino acid use.

It is possible that if impaired intestinal absorption resulted in increased amino acid degradation in the caecum and an increased proportion of N absorbed in the form of ammonia that a lower efficiency of utilisation of apparently digested N would ensue.

An increased irreversible loss of urea N has been observed in sheep infected with T. colubriformis (Roseby and Leng, 1974) but this does not differentiate between ammonia from amino acid degradation within the body and within the alimentary tract. Roseby (1977) concluded that the combined effects of increased leakage of endogenous protein into, and reduced efficiency of absorption from, the small intestine resulted in a reduction in the net absorption of amino acid N by infected sheep. The corresponding increase in microbial deamination of protein in the large intestine increased the availability of ammonia, but very little of this ammonia was used for amino acid synthesis as most of this was converted to urea and excreted.

Sykes and Coop (1976b) showed that in the infected animal

the deposition of fat was severely impaired and the efficiency of utilisation of metabolisable energy was reduced. It is not clear whether this was due to a change in maintenance requirement or in efficiency of fattening.

Sykes and Coop (1976b) reported that the mineral balances showed that both net Ca and P retention by the infected group were inferior to those of the pair-fed controls. Reveron et al (1974a) showed that the metabolism of P was more adversely affected than that of either Ca or magnesium by an infection of T. colubriformis in growing lambs.

EFFECTS ON ENDOCRINE RESPONSES

Prichard et al (1974) showed that corticosteroid levels rose for both infected and pair-fed sheep, the increase being much greater in infected animals, that insulin was depressed in both infected and pair-fed animals to a similar degree when feed intake fell and that thyroxine levels fell in infected sheep, whereas pair-fed animals showed no change. This could be the reason for the changes in distribution of protein synthesis between the liver and musculature in acute intestinal infections as elevated corticosteroid concentrations are known to have a catabolic effect on muscle protein and an anabolic effect on liver protein. Insulin tends to promote muscle protein synthesis and to increase liver protein catabolism, and thus the depression in insulin could be expected to impair muscle protein synthesis and to reduce protein catabolism in liver (Symons and Jones, 1971; 1972).

Increased urinary N excretion has been demonstrated as a

result of infection with T. colubriformis (Roseby, 1973) and this effect could be due to the stimulation by the parasite of corticosteroid production.

EFFECTS ON THE SKELETON

Sykes and Coop (1976b) showed that there was a poor retention of Ca and P, thus demonstrating that T. colubriformis has a dramatic effect on the mineral metabolism of the host, thus supporting the conclusions of Reveron et al (1974a) who found certain bone deformities and abnormalities.

Sykes et al (1975) showed that bone size and bone quality were severely reduced, the latter because of reductions in the density of bone matrix and in its degree of mineralisation. They also found that the predominant lesion was osteoporosis - rarefaction of cortical and cancellous bone - with severe retardation of epiphyseal bone growth, cross bridging of trabeculae and marked absence of osteoblasts in most severely affected bone. Sykes et al (1975) and Coop et al (1976) argued on the basis of changes in blood chemistry and detailed chemical and histological examination of individual bones than an induced Ca and P deficiency was responsible.

However, in view of the postulated changes in corticosteroid production the possibility of a concurrent hormonally induced matrix - osteoporosis should not be excluded (Sykes et al, 1975).

EFFECTS ON DIGESTA FLOW AND FERMENTATION

Roseby (1977) reported that the flow rates from the rumen

were slower in the sheep infected with T. colubriformis. He also observed that the dry matter, water and N contents of the rumen were decreased, while those of the abomasum, small intestine and caecum-proximal colon were increased, in infected sheep.

Roseby (1977) reported that there was no change in rumen volatile fatty acid (VFA) concentration. Roseby (1973) had previously observed that T. colubriformis parasitism did not affect the total VFA concentration in rumen fluid sampled via cannulae. However, as the rumen fluid volume in infected sheep was less than in the controls, the rate of production of VFA in the rumen may have been less, as observed by Steel (1972). Ammonia concentration and pool size were both lower in the rumens of infected sheep. In the caecum - proximal colon both VFA and ammonia concentrations and pool sizes were higher in the infected sheep, probably reflecting increased fermentation due to increased flow of fermentable substrate from the small intestine.

DIAGNOSTIC AIDS

Anthelminthic treatment at three weekly intervals is cost effective if it prevents only 8% loss of animal efficiency, but such small differences in food utilisation are difficult to detect as weight change.

Diagnostic evidence can be obtained from faecal egg counts but this is not very reliable as was confirmed by Reveron et al (1974b), Coop et al (1976; 1977). It is, therefore, logical that blood parameters should also be examined for correlated changes as aids to diagnosis. Ideally they

should be specific and quantitatively related to metabolic damage caused. Those currently measured fall into three categories, indicating (a) damage to a particular site, (b) invasion by foreign organisms, eg. leucocyte counts or immunoglobulins and (c) the metabolic consequences of the interactions between parasitic damage and the nutritional and physiological state of the host.

(a) Elevated plasma pepsinogen concentrations have been shown to indicate abomasal damage (Coop et al, 1977). Its value as a semi-quantitative index of consequential metabolic derangement has not been fully evaluated but a relationship between growth rate and abomasal pH (Thomas and Waller, 1975) and reductions in growth rate and feed efficiency in association with values above 1000 mU/l tyrosine/l/min (Coop et al, 1977) have been reported in chronic ostertagia infections.

(b) The immune responses are unlikely to provide quantitative data on the effect of parasitism on host metabolism, though their sensitivity may ultimately aid detection on sub-clinical infections.

(c) Serum albumin concentration is sensitive to sub-clinical parasitism of the gastro-intestinal tract (Holmes and MacLean, 1971; Barker, 1973) though part of the response will be to maintain fluid osmotic pressure in hyperglobulinaemia (Coop et al, 1976). The quantitative significance cannot yet be assessed, but a nutritional hypoalbuminaemia would be distinguished by a low plasma urea N concentration

(Sykes and Field, 1973).

Hypophosphataemia in sub-clinical intestinal parasitism may aid diagnosis in young growing lambs (Coop et al, 1976) although susceptibility to dietary and other influences indicates lack of specificity.

CALCIUM AND PHOSPHORUS METABOLISM

The major pathways of Ca and P metabolism are intake, absorption, transport within the body to various sites, deposition in and removal from bone and excretion in faeces and urine.

ENTRY OF CALCIUM AND PHOSPHORUS INTO THE GASTRO-INTESTINAL TRACT

These elements enter the digestive tract in the food and digestive secretions; for Ca the more important source is the former and for P the latter.

Dietary intake of calcium and phosphorus

This is determined by the intake and concentration of Ca and P in the animal's diet.

Roughages are commonly rich in Ca and poor in P, whereas concentrates and cereals show the reverse relationship. The concentrations of Ca and P in roughages fall typically in the range 2-10 and 1-5 g/kg DM respectively. The corresponding values for concentrates are 0.2-2 and 2-12 g/kg.

The concentration of Ca and P in herbage is of particular relevance in the present context as herbage forms the basic diet of cattle and sheep and the grazing ruminant is

exposed to parasitic infection. In any one area, various plant types will be present in the sward, and these types may show wide variation in their Ca and P contents. Thus, Thomas et al (1952) reported that the Ca contents of lucerne, sanfoin, perennial ryegrass and chewings fescue grown in the same environment were 21.5, 12.3, 4.6 and 2.4 g/kg DM respectively, while the corresponding P contents were 3.9, 3.8, 2.5 and 1.9 g/kg DM.

Many factors influence the concentration of Ca and P in herbage. The botanical composition of any area is largely determined by the nutritive state of the soil, and by conditions of moisture and temperature. Hill (1962) pointed out in his review that these environmental factors also influence the Ca and P contents of any individual plant species. The age of the herbage influences its Ca and P content. P concentrations show marked falls with advancing maturity of the plant, whereas the effect on Ca is variable (Hill, 1962).

The amounts of Ca and P ingested by grazing animals are only partly influenced by these factors, because these animals have selective grazing habits. Due to an increased concentration of P in the meristematic tissues, larger quantities are found in the young, developing leaves. This, coupled with species differences, presents difficulties in assessing the P concentration in herbage selected by the grazing animal. The closer grazing of younger leaves by sheep may account for the observation that they are apparently less susceptible to aphosphorosis than cattle

when both species graze the same area.

Numerous studies have been made of the factors determining the feed intake of ruminants. These include such animal characteristics as species, weight and physiological state, such feed characteristics as energy value, and such environmental factors as day length and temperature. The Agricultural Research Council (ARC) (1980) has reviewed existing information and have concluded that the DM intake of coarse diets (including herbage) of growing lambs is highly dependant upon the energy content of the diet and can range from 0.31 kg/d for 20 kg animals on low energy diets to 1.65 kg/d for 60 kg animals on high energy diets. The corresponding figures for growing cattle were 2.1 kg/d for 100 kg animals to 12.0 kg/d for 600 kg animals.

Combining the information on the DM intake and concentration of Ca and P in herbage it can be predicted that the Ca and P intakes of grazing lambs will vary typically within the range 2-14 and 1-7 g/day respectively.

SECRETION OF CALCIUM AND PHOSPHORUS INTO THE DIGESTIVE TRACT

Saliva

Ruminants secrete large volumes of alkaline and well - buffered saliva. This not only moistens the food in the mouth, aiding chewing and swallowing, but also stabilises the rumen pH and supplies most of the water and salts of the rumen fluid. Sheep are estimated to secrete 6-16 litres of saliva daily (Kay, 1960). The parotid glands, which flow continuously, contribute about half of this daily

flow. Ruminants have a unique ability to transfer very large quantities of material from blood to gut via the salivary secretions.

Ruminant saliva contains very high concentrations of inorganic P and very low concentrations of Ca (McDougall, 1948; Storry, 1961). In sheep, McDougall (1948) estimated the average concentration in mixed saliva to be 0.4 - 0.7 mg/ml. Inorganic P concentrations in parotid and mixed saliva are higher than that in serum by a factor of 5 to 19 for sheep (McDougall, 1948; Tomas et al, 1967).

P output in saliva is a function of both flow and concentration and is, therefore, subject to the many variables affecting both, as well as to the large variation amongst animals. A factor which has the most important effect on daily salivary flow rates is the physical nature of the diet fed. Salivary flow rates decrease if the diet is pelleted (Putnam et al, 1966), if the dry matter intake is reduced (Wilson and Tribe, 1963), if the diet is finely ground (Wilson and Tribe, 1963) or if concentrate diets are fed (Bailey, 1961). These findings may be explained on the grounds that these diets reduce the animal's time spent eating and ruminating and thus lessen the reflex stimulation of the salivary glands (Coats et al, 1956).

For any given type of diet, P output in saliva is dependent on P intake, (Denton, 1956; Tomas et al, 1967) and salivary P concentration is directly related to plasma P concentration (Watson, 1933a; Tomas et al, 1967). Coats and Wright (1957) and Kay (1960) using sheep, repo rted that although the

inorganic P concentration decreased as the saliva flow rate increased, the total salivary P secretion increased. Thus on diets requiring high salivary flow rates, the daily salivary P secretion will also be high. Bailey and Balch (1961) reported that total salivary P secretion was directly related to salivary flow rate.

There are no direct measurements of the total salivary P, but using a salivary flow of 8-12 l/day and a concentration of inorganic P in mixed saliva of 0.4-0.7 g/l (McDougall, 1948) it can be calculated that sheep secrete 3-8 g of inorganic P in the saliva per day.

Clark (1953) reported that concentrations of soluble P in rumen digesta did not fall below 200 mg/l, even in the presence of clinical aphosphorosis. He suggested that rumen inorganic P levels are determined by the salivary P levels, and are independent of feed P levels.

A number of papers have appeared which would seem to invalidate this claim (Preston and Pfander, 1964; Tomas et al, 1967). They reported that rumen P concentration was related to dietary P intake in a positive manner. However, Tomas (1973) has concluded that salivary P is the most important determinant of soluble P levels in the rumen. He attributes the wide variation in rumen P levels reported in the literature to differences in solubility of dietary P.

The large amounts of P in ruminant saliva may be necessary to ensure an adequate supply of P for the rumen microorganisms (Tomas et al, 1967; Tomas, 1973). Field et al (1975) found that a simple dietary P deficiency lowered

DM digestibility and suggested that the level of P in the ruminal fluid was inadequate for the cellulose splitting organisms.

Secretion of calcium and phosphorus into other areas of the gut

The transfer of P across the rumen wall in the sheep is negligible (Scarisbrick and Ewer, 1952; Parthasarathy et al, 1952; Sperber and Hydén, 1952; Wright, 1955a; Hydén, 1961).

Garton (1951) found the concentration of inorganic P in gastric juice of sheep to be 0.2 mg/100 ml. Ash and Kay (1963) reported that the abomasum of the sheep secreted 2-8 litres/day of gastric juice, therefore implying a daily secretion of P into the abomasum of only 4-16 mg.

Kay (1969) reported that secretions collected from an isolated intestinal loop in a conscious sheep (including bile, pancreatic juice and duodenal secretions) contained 0.09 g/day of inorganic phosphate.

Very little P is secreted into the intestines (Smith et al, 1955; Annenkov et al, 1970). However, Ben - Ghedalia et al (1975) reported a net secretion of P in the last 10 metres of the small intestine of sheep, amounting to 1.4 g/day.

Apart from the small amount entering in the saliva endogenous Ca reaches the gut via other digestive secretions. Storry (1961) showed that significant amounts of Ca entered the abomasum in the gastric secretions, and the duodenum in the biliary secretions. Care and Van't Klooster (1965)

also found that there was net secretion of Ca into the abomasum and duodenum.

ABSORPTION OF CALCIUM AND PHOSPHORUS

Site of absorption

There is no doubt that the major site for Ca and P absorption in the ruminant is the small intestine (Phillipson and Storry, 1965; Pfeffer *et al*, 1970; Grace *et al*, 1974; Ben - Ghedalia *et al*, 1975). Recent studies have shown the upper small intestine as the main site of Ca and P absorption within the small intestine with absorption being most active in a region 1-3 metres from the pylorus (Ben - Ghedalia *et al*, 1975).

It is interesting to relate the area of absorption of Ca and P with that damaged by *T. colubriformis*. Studies on the distribution of mucosal abnormality caused by *T. colubriformis* within the small intestine has shown that the main area affected is the first six metres with the greatest damage occurring within the first three metres (Barker, 1974).

Mechanism and control of absorption

The mechanism of Ca absorption in non-ruminants involves two processes, a non-saturable diffusional one, related to intestinal Ca concentration, and a saturable active one, independent of concentration but related to body needs (Wasserman and Taylor, 1969). These two processes probably also operate in ruminants.

A change in intake would result in an immediate and

corresponding change in the diffusional component, and thereafter total absorption and adaptation to the new intake would then be due to an alteration in the active component (Wasserman and Taylor, 1969). It is generally accepted that Vitamin D is involved in the mechanism of active absorption of Ca and that dihydroxycholecalciferol is the active metabolite (De Luca, 1974; 1975). Although little is known of the mechanism of phosphate absorption, recent studies in non-ruminants suggest an active transport system, different from that involved in Ca transport, but also stimulated by Vitamin D (Harrison and Harrison, 1961; Wasserman and Taylor, 1973; Taylor, 1974). Chen et al (1974) reported that the phosphate system was responsive to dihydroxycholecalciferol, the active metabolite of Vitamin D.

Since absorption of Ca in adult ruminants appears to increase to a high level only after the skeletal reserves of Ca have been depleted and to return to normal only when the reserves have been replenished, control according to skeletal reserves of Ca is indicated (Braithwaite et al, 1969; 1970; Braithwaite, 1974; Sykes and Dingwall, 1975). However in spite of considerable evidence from work on ruminants and non-ruminants, no mechanism by which demineralised bone might control Ca absorption has yet been established. De Luca (1974; 1975) proposed that a change in the Ca requirements of bone would set in motion a chain of events involving changes in the plasma Ca concentration, the relative concentrations in plasma of parathyroid hormone and calcitonin, the rate of secretion

of dihydroxycholecalciferol and finally the rate of Ca absorption.

Morrissey and Wasserman (1971), however, observed that Ca absorption in non-ruminants did not correlate with plasma Ca and Braithwaite et al (1969; 1970) observed, in pregnant and lactating sheep, that Ca absorption did not increase immediately in response to increased demands, but reached a high level only in mid-lactation when substantial reserves of skeletal Ca had already been mobilised.

Conrad et al (1956) reported that large doses of Vitamin D administered to cows markedly enhanced the absorption of dietary Ca and P. This effect of Vitamin D on phosphate absorption is probably due to dihydroxycholecalciferol the active metabolite of Vitamin D (De Luca and Schnoes, 1976) although Wasserman and Taylor (1976) have challenged this view.

Efficiency of absorption of dietary calcium and phosphorus

To convert net requirements into dietary requirements one must be able to predict accurately the amount of a nutrient absorbed from the amount ingested. At present there is no convincing evidence that this basic premise holds for ruminants with respect to Ca. Dietary Ca is absorbed according to body needs (Lueker and Lofgreen, 1961; Young et al, 1966b). At a given intake, absorption of dietary Ca depends on the net requirements of the animal and, at a given net requirement, the coefficient of absorption varies inversely with feed Ca. Visek et al(1953a)

and Braithwaite and Riazuddin (1971) observed that adult male animals, irrespective of intake, absorbed sufficient Ca only to replace that lost by excretion into urine and intestine.

Braithwaite and Riazuddin (1971) and Braithwaite (1975b) reported that young animals with high growth requirements absorbed and retained Ca in direct relation to intake over a wide range of Ca intakes. Braithwaite (1975b) found that above dietary intakes of 200 mg/d per kg body-weight for mature animals and above 400 mg/d per kg body-weight for young animals, no significant increase in Ca absorption occurred.

Braithwaite et al (1969) and Sykes and Dingwall (1975) established, using sheep, that absorption was related positively to requirements and negatively to Ca intake. Ca absorption is increased after a period of dietary Ca deficiency, but a substantial loss of body stores of Ca appears to be necessary before this increase occurs (Braithwaite, 1974; 1975b; Sykes and Dingwall, 1975). Braithwaite et al (1972a) reported that virtually all the Ca absorbed in excess of the small amount required to replace the unavoidable excretion is retained by growing animals.

During pregnancy and lactation Ca absorption is increased in adult animals (Braithwaite et al, 1969; 1970). However, certain cows, cannot absorb sufficient Ca to meet their requirements for milk production, and secondly, the Ca

intake of dairy cows in the dry period has a bearing on the subsequent incidence of parturient hypocalcaemia and milk fever. This has been summarised by Duncan (1958) who, using data from published balance trials, has shown an association between high milk yields and negative Ca balance. Thus it must be appreciated that certain dairy cows, particularly in early lactation, will be in negative Ca balance, irrespective of the Ca content of the diet, and that resultant losses from the skeleton will be made up in the subsequent dry period. There is some evidence that the same situation holds for lactating ewes (Braithwaite et al, 1969).

Dietary P is absorbed by ruminants in direct relation to the P intake (Tillman et al, 1959; Preston and Pfander, 1964). In contrast with Ca, Young et al (1966c) reported that P is absorbed in excess of requirements and the extra excreted. ARC (1980) looked at data on the absorption of P from diets fed to sheep. They divided the data into two groups according to age, above and below one year and found that within each age class the linear relationship between absorption and intake was highly significant ($P < 0.001$) and accounted for 85-90% of the variation in absorption. The estimates for the absorption of dietary P were 0.73 for lambs up to one year of age and 0.60 for more mature sheep.

Field, Dingwall and Munro (personal communication) using sheep, have measured the absorption of salivary P and found it varied between 0.75 and 0.95. Salivary P is the

main component of digestive secretions. They also found that the absorption of digestive secretions was always higher than the corresponding value for the absorption of dietary P.

Dietary factors affecting availability of calcium and phosphorus

Dietary Ca - binding substances such as oxalate and phytate, which depress Ca availability in non-ruminants, are less effective in ruminants because they are metabolised by micro-organisms in the rumen (Reid et al, 1947; Talapatra et al, 1948). However Talapatra et al (1948) reported that the ruminal microflora converted the oxalates into alkaline bicarbonates and carbonates and thus Ca absorption was indirectly upset by the increased pH. Smith et al (1968) reported that nucleic acids, which are formed in the rumen and pass largely unchanged into the duodenum, were potent Ca - binding substances.

Visek et al (1953b) reported that dietary protein had no effect on Ca absorption in dairy cattle, but Sykes and Field (1972) observed that Ca retention was decreased in pregnant sheep given a low protein diet.

The availability of dietary Ca is reduced when fat is added to the diet (Tillman and Brethour, 1958b). Chicco et al (1973) observed that large amounts of dietary magnesium had a similar effect on Ca availability. Ben - Ghedalia et al (1975) reported that the solubility of Ca in the gastro-intestinal tract was related to the pH of the digesta. Ca absorption is favoured by a low pH (Ender and Dishington, 1970). Wasserman and Taylor (1969) reported

that grain added to diets promoted Ca absorption and that this might be due to a decreased intestinal pH resulting from the products of digestion from the grain.

Tillman and Brethour (1958a) reported that inorganic P and phytate P were equally well absorbed.

P availability, like that of Ca, may be affected by other constituents of the diet. Mudgal and Ray (1967) observed improvements in P retention in calves when dietary protein levels were increased, and Leibholz (1974) found the P availability was reduced in sheep given a low energy diet. Martin (1963) reported that magnesium deficiency led to an increased absorption and retention of P, while sulphur, although decreasing the absorption of P, increased the retention of that which was absorbed. No mechanism for the effects of magnesium and sulphur are known.

Lueker and Lofgreen (1961) observed that ruminants could tolerate a wide range of Ca:P ratio in the diet without affecting the absorption of either. However Chen et al (1974) reported that at times of high demand large ratios could be harmful. Wise et al (1963) reported that a large excess of Ca over P depressed the growth of cattle. Tomas (1968) postulated that a function of saliva was to regulate the Ca:P ratio of diet.

Young et al (1966b) suggested that the tolerance of ruminants, unlike non-ruminants, to wide ratios of Ca:P, was related to the pH at the site of absorption in the upper small intestine. Since the upper small intestine

of the sheep is more acidic than in man, the precipitation of P as tricalcium phosphate will therefore be reduced and the Ca and P availability increased.

Requirements of calcium and phosphorus

The factorial approach which assesses requirements in two stages has been used to estimate the mineral requirements of animals of different classes producing at different rates. Firstly, the net requirement is calculated from estimates of the storage and secretion of the element made during growth, pregnancy and lactation and of inevitable losses from the body (endogenous losses). Secondly, the dietary requirement is calculated by dividing the net requirement by a factor that represents the proportion of dietary material that is absorbed as assessed from the balance trials. In detail:

Net minimum endogenous requirement (E)	=	the inevitable loss of the element from the body in faeces and urine.
Net requirement for body growth (G)	=	daily retention of the element at the specified rate and stage of growth.
Net requirement for pregnancy (P)	=	daily retention of the element in the foetus at the specified stage of pregnancy.
Net requirement for lactation (L)	=	daily secretion of the element in milk at the specified yield.
Total net requirement	=	$E + G + P + L$
Dietary requirement, where A is the coefficient of absorption	=	$(E + G + P + L)/A$

Absorption is defined as the amount of a mineral supplied in the diet that enters the body from the gut. The

coefficient of absorption (or of apparent absorption) is the amount absorbed (or apparently absorbed) divided by the amount ingested. In theory, the factorial method provides a satisfactory basis for the assessment of dietary requirement.

In ARC (1965) a figure of 40 mg/kg live weight per day was adopted as the total minimum endogenous loss for Ca. Since that date extensive work has shown that the endogenous loss is much less than previously thought for sheep. Field and Suttle (1969) gave low Ca diets to growing lambs and mature wethers and found that faecal endogenous losses probably lay in the range of 11-13 with a maximum of 22 mg/kg per day. Hodge (1973a) found a value of 17.7 after 32 days on the calcium - depleted diet and one of 8.8 mg/kg per day after 32 to 64 days on the diet. ARC (1980) adopted a figure of 16 mg/kg live weight per day for total endogenous loss of Ca for sheep. For the absorption of dietary Ca, they adopted a figure of 0.68 and 0.95 for sheep and milk-fed lambs respectively.

In ARC (1965) the endogenous faecal excretion of P was taken as a constant and equal to 42 mg/kg live weight per day for sheep. Observations on the faecal output of phosphorus by sheep consuming experimental or natural diets low in P demonstrated that this value for endogenous faecal loss was a gross over-estimate (Field et al, 1974; Sykes and Dingwall, 1976). For instance, faecal excretions of 12-14 mg/kg per day have been reported by Sykes and Dingwall (1976). ARC (1980) adopted a figure of 14 mg/kg

liveweight per day for the total endogenous loss of P for sheep. For the absorption of dietary P, they adopted a figure of 0.73, 0.60 and 0.95 for lambs up to a year of age, for more mature sheep and for milk-fed lambs respectively.

As this study was concerned with the effects of parasitism on growing lambs, only the Ca and P requirements for growing animals, as recommended by the ARC (1980), are given in Table 1.1.

The dietary requirements of Ca and P for growth as a percentage of the total growth requirements are shown in Table 1.2. The rate of gain has a large effect on the Ca and P requirements for growth. As the rate of growth increased from 0.1 to 0.4 kg/day for a 20 kg animal, the growth requirements for Ca and P increased from 38 to 89% and 12 to 62% respectively.

CALCIUM AND PHOSPHORUS IN THE BLOOD

In normal adult animals, the serum inorganic P level is of the order of 4-7 mg/100 ml, while the normal range for Ca is 9-12 mg/100 ml. Marsh and Swingle (1955) and Hackett et al (1957) found that sheep fitted this pattern for both inorganic P and Ca. Variation in plasma Ca in any one animal is very narrow, because plasma Ca is very precisely controlled (Rasmussen and De Luca, 1963) but plasma inorganic P is far more variable (Simesen, 1963). Watson (1933c) reported that most of the total blood P in the sheep was in the organic form, as is true for other animals.

Both Ca and inorganic phosphate exist in plasma as (a) protein-bound, (b) ionised and (c) complexed, only the ionised and complexed forms are ultrafiltrable (Walser, 1960; 1961; Robertson and Peacock, 1964). Robertson and Peacock (1964) estimated in humans that 40.9% of the Ca was protein-bound, 50.8% was ionised and 8.3% was complexed. Therefore only about 60% of the plasma Ca can be filtered at the glomerulus. These workers also estimated that 4% of the inorganic phosphate was protein-bound and 96% was ultrafiltrable. Walser (1960; 1961) obtained similar results in humans for Ca but he found that 12% of the inorganic phosphate was protein-bound and 88% was ultrafiltrable.

EXCRETION OF CALCIUM AND PHOSPHORUS

Ca and P are lost from body tissues by excretion into the intestine and in the urine. Losses into the intestine occur largely in the digestive juices and although the excreted Ca and P might be expected to mix with dietary Ca and P and be reabsorbed at the same efficiency, a lack of correlation between endogenous Ca in the faeces and the Ca intake or total faecal Ca suggests that some discrimination occurs (Ramberg et al, 1970).

The rate of excretion of endogenous faecal Ca increases with increasing age, but remains fairly constant when body weight is taken into account (Visek et al, 1953a; ARC, 1965; Braithwaite and Riazuddin, 1971). Visek et al (1953a) reported that the rate of excretion of endogenous faecal Ca was not affected by the amount of Ca ingested or

absorbed. However, Braithwaite (1974) observed that the rate of excretion of endogenous faecal Ca decreased slowly when animals were fed a Ca - deficient diet for a prolonged period.

The rate of excretion of endogenous faecal P is directly related to P intake and rate of absorption (Kleiber et al, 1951; Lueker and Lofgreen, 1961; Preston and Pfander, 1964; Young et al, 1966c) and is inversely related to the rate of Ca absorption (Lueker and Lofgreen, 1961). Preston and Pfander (1964) considered that the rate of endogenous faecal P excretion was related to the plasma inorganic P concentration and Young et al (1966c) suggested that it played a part in P homeostasis. Clark et al (1973) observed that endogenous faecal P increased after intravenous infusion of P and they deduced that this excretion occurred via the salivary glands.

The Ca content of ruminant urine is generally low (ARC, 1965). Braithwaite (1974; 1975b) and Braithwaite and Riazuddin (1971) reported that urinary excretion of Ca was unaltered by changes in the rate of Ca intake or absorption. Non-ruminants absorb more Ca than they need and excrete the excess in the urine (Bronner, 1973). Young et al (1966c) reported that, in ruminants, P deficiency caused an increase in urinary excretion of Ca. Braithwaite (1972) observed that urinary excretion of Ca increased after the ingestion of the acidic substance ammonium chloride.

Urinary P excretion is normally low (Preston and Pfander,

1964; ARC, 1965; Manston and Vagg, 1970) but occasionally an individual animal will give a high value (Manston and Vagg, 1970). Braithwaite (1975b) reported that urinary P excretion tended to be higher with increased P intake. Walker (1972) observed that excretion was also high in animals receiving Ca deficient diets but this excretion decreased as the Ca intake was increased (Hodge, 1973a; 1973b; Braithwaite, 1975b). Braithwaite (1975b) concluded that the changes in P excretion occurred as a result of changes in bone metabolism.

Ruminants excrete significant amounts of urinary P during starvation (Watson, 1933b; Dale et al, 1954) and when they are being fed diets containing significant amounts of concentrates. There are two broad groups of concentrate diets, those causing an acidosis (Reed et al, 1965; Topps et al, 1966) and those which lead to the problem of urolithiasis in sheep (Bushman et al, 1965; Robbins et al, 1965; Bushman et al, 1967; Crookshank et al, 1967). There is a direct relationship between plasma inorganic P, urinary P and the incidence of urinary calculi. It would seem that a rise in plasma inorganic P is important, both for starvation and the feeding of concentrate diets.

SKELETAL METABOLISM OF CALCIUM AND PHOSPHORUS

The skeleton contains 99% of the total body Ca and 80% of the total body P and the relative proportion of these elements remains fairly constant (ARC, 1965; Field et al, 1975). Therefore changes in retention of Ca and P result from changes in the rates of bone accretion or resorption.

Braithwaite and Riazuddin (1971) reported, using sheep, that the rate of accretion of Ca into bone was high in young animals, but decreased to a low level at maturity. When the animal is growing, the rate of bone accretion is greater than the rate of bone resorption but when the animal reaches maturity the two processes become approximately equal. Both processes then decline as the animal becomes older.

Braithwaite (1975b) and Braithwaite and Riazuddin (1971) reported that bone resorption was the main pathway governing Ca homeostasis as bone accretion remained constant in animals of a given age. Recently it has been shown by Braithwaite (1975b) that maximum skeletal retention of Ca occurred when the rate of bone resorption became zero and was approximately equal to the rate of bone accretion. Since accretion decreases with age (Braithwaite and Riazuddin, 1971) so also will the maximum rate of retention.

The skeletal reserves of Ca and P of ewes are lost during pregnancy and early lactation and these reserves are replenished in late lactation and the ensuing dry period by changes in the rate of bone resorption (Benzie *et al*, 1959; Braithwaite *et al*, 1969; 1970).

The rate of accretion however, increases in early lactation in both sheep and dairy cattle, possibly to increase the maximum rate of retention of Ca and to ensure that the lost reserves are replaced quickly (Braithwaite *et al*, 1970; Ramberg *et al*, 1970; Braithwaite, 1975b).

Benzie et al (1959) reported that a low P intake caused resorption of the skeleton during pregnancy and lactation and skeletal reserves were replenished when demands for P fell during late lactation.

Only a small fraction of the Ca and P of bone is rapidly exchangeable with the ionic Ca and P of blood and soft tissues. Braithwaite and Riazuddin (1971) reported that the exchangeable Ca pool of sheep decreased in size with age and that the size was directly related to the rate of Ca accretion into bone. Ramberg et al (1970) considered that the exchangeable pool of Ca must play some part in supplying the additional requirement for Ca as the pool decreased in size at the onset of lactation. Young et al (1966a) reported that the exchangeable Ca pool was reduced in size in P depleted sheep to approximately 50% of that in the controls.

ENDOCRINE REGULATION OF CALCIUM AND PHOSPHORUS METABOLISM

Parathyroid hormone is secreted by the parathyroid gland in response to hypocalcaemia and stimulates the conversion of 25 - hydroxycholecalciferol to 1, 25 - dihydroxycholecalciferol (Fraser and Kodicek, 1973). Bone resorption is stimulated by parathyroid hormone in the presence of 1, 25 - dihydroxycholecalciferol and intestinal absorption is stimulated by 1, 25 - dihydroxycholecalciferol alone (De Luca, 1974; 1975). When the Ca levels in the blood become too high calcitonin is secreted by the thyroid gland and parathyroid hormone secretion is decreased. The increased calcitonin concentration blocks bone resorption

(Taylor, 1972) and the decreased parathyroid hormone concentration depresses Ca absorption.

P absorption is stimulated by 1, 25 - dihydroxycholecalciferol (Chen et al, 1974). P deprivation results in a stimulation of 1, 25 - dihydroxycholecalciferol synthesis and the mobilisation of bone mineral (De Luca, 1974; 1975).

Tomas (1974) demonstrated that parathyroid hormone increased the P concentration in saliva and thereby stimulated its secretion. In anaesthetised sheep, he observed a rapid dose-related increase in P output by the parotid gland when parathyroid hormone was infused intravenously. Furthermore, direct infusion of small amounts of parathyroid hormone into salivary glands demonstrated that this occurs at physiological levels (Tomas, 1974). These results have been confirmed for conscious sheep (Clark et al, 1975).

Braithwaite (1975a) reported that administration of growth hormone to nearly mature sheep resulted in increased rates of Ca absorption, Ca retention and bone accretion and an increased size of the exchangeable Ca pools. Braithwaite et al (1972b) reported that administration of oestrogen to wether lambs, aged six to nine months, resulted in a similar pattern of Ca metabolism and they suggested that oestrogen and growth hormone promoted increased growth and Ca retention in ruminants by altering the metabolism of Ca to that of a younger more actively growing animal.

OUTLINE OF RESEARCH PROGRAMME

Nematode parasites, resident in the gut of sheep, pose serious problems to the livestock industry. Parasites are known to have harmful effects on the host animal and these adverse effects appear to be due to biochemical and physiological disturbances of the sheep's metabolism. The nature of the adverse effects has been sought through studies of the pathological lesions, clinical effects and blood composition changes accompanying infection.

Most of the recent work has been focussed on the effect of chronic parasitism on food intake, growth rate, faecal egg production, plasma biochemistry and digestibility and utilisation of food, using O. circumcincta (Coop et al, 1977, Sykes and Coop, 1977) and T. colubriformis (Coop et al, 1976; Sykes and Coop, 1976b). The work on food digestibility and utilisation was mainly concerned with apparent N and energy digestibility, efficiency of energy utilisation and N and fat deposition.

Chronic sub-clinical parasitism of the abomasum and the proximal small intestine of lambs had also been shown to influence mineral metabolism and impair skeletal growth (Sykes et al, 1975, 1977). However, in these studies the relative contributions of dietary and endogenous losses of Ca and P to the faecal outputs of each animal were not determined. The measurement of endogenous faecal Ca and P is important for knowledge of an animal's Ca and P metabolism.

The object of the work in this thesis was to investigate

the effects of O. circumcincta and T. colubriformis on the Ca and P metabolism of growing lambs. Radioactive Ca and P were used to measure the endogenous faecal loss of these elements. In order to establish the rate of flow of digesta and also the rate of flow of Ca and P through the gastro-intestinal tract the animals were cannulated at the rumen, abomasum and ileum and the radioactive markers ^{51}Cr - EDTA and (^{103}Ru (phen)$_3$) Cl$_2$ were used.

Two series of experiments were carried out:

(i) The first series of experiments were designed as a preliminary investigation mainly to assess various techniques for studying the changes in metabolism of Ca and P. Where possible, the effects of O. circumcincta and T. colubriformis on the metabolism of Ca and P were also assessed.

(ii) Using the experimental techniques decided upon after the first series of experiments, the objects of the second series of experiments were to investigate the effect of the two internal parasites on (a) performance and certain plasma constituents, (b) the absorption and utilisation of Ca and P from the diet and (c) the flow of digesta and the flow of Ca and P along the gastro-intestinal tract.

TABLE 1.1 Dietary requirements (g/day) of calcium and phosphorus for sheep gaining at different rates

LIVE WEIGHT (kg)	RATE OF GAIN (kg/day) 0		0.1		0.2		0.3		0.4	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
5	0.1	0.1	1.6	0.8	3.1	1.6	4.6	2.4	6.1	3.1
10	0.2	0.2	1.7	0.9	3.2	1.7	4.7	2.5	6.2	3.2
20	0.5	0.4	2.0	1.1	3.4	1.9	4.9	2.6	6.4	3.4
40	0.9	0.8	2.4	1.5	3.9	2.3	5.4	3.0	6.9	3.8
60	1.4	1.2	2.9	1.9	4.4	2.7	5.9	3.4	7.3	4.2

TABLE 1.2 The dietary requirements of calcium and phosphorus for growth as a percentage of the total growth requirements for sheep

LIVE WEIGHT (kg)	RATE OF GAIN (kg/day) 0.1		0.3		0.4	
	Ca	P	Ca	P	Ca	P
20	38	12	82	48	89	62
40	27	8	72	35	80	48
60	23	7	65	29	75	40

The values in Table 1.2 were calculated using the minimum endogenous losses of Ca + P.

CHAPTER 2

MATERIALS AND METHODS

This chapter describes the materials and methods common to all experiments: establishment of parasitic infection, measurement of endogenous and exogenous components of digesta and faeces, flows of digesta along the gut, measurement of radioactivity and chemical composition of samples.

ESTABLISHMENT OF PARASITIC INFECTION OF OSTERTAGIA CIRCUMCINCTA AND TRICHOSTRONGYLUS COLUBRIFORMIS

The parasitic infection was induced by giving the lambs continuous small doses of infective larvae (ie trickle type of infection). This dosing regime was chosen because it models the larval intake of a grazing animal more closely than does a single large dose, commonly used in the past. Furthermore, it is difficult to establish a 'steady state' type of infection necessary for the present studies with a single large dose because the animal either succumbs, becomes clinically affected or recovers. Sykes and Coop (1976a) have generated in growing lambs, with trickle dosing, parasitic infections which have resulted in the animals having constant but reduced appetites, impaired efficiency of skeletal mineralisation and low efficiency of food conversion.

The third stage infective larvae were prepared from faeces obtained from animals with pure infestations of T. colubriformis or O. circumcincta. With faeces kept at a constant temperature of 21 °C, the optimum period for

the development of the larvae to the third stage is ten days. The larvae were extracted by a modification of the method of Christie and Patterson (1963). Infective larvae were stored at 4°C. The chosen number of larvae were given daily in 10 ml of water by the oral route. The establishment of infection was monitored as follows:

Egg counts

Egg counts were recorded weekly from week three of infection onwards. Faeces were collected from the rectum and the number of eggs per gram (epg) estimated by a modification of the method of Jackson (1974).

Pepsinogen

Reid and Armour (1975) considered pepsinogen estimations to provide a useful guide to the degree of abomasal damage present in grazing sheep. Infection with O. circumcincta causes an elevation of plasma pepsinogen levels. Blood samples were taken at regular intervals and analysed for pepsinogen.

Slaughter procedure

At the end of the observational period the lambs were killed with sodium pentobarbitone and the small intestine was immediately removed, washed and digested in pepsin/HCl according to procedures used by Coop and Angus (1975). Washings and digests were passed through a 400 mesh sieve and the material retained made up to two litres and formolised. Worm burdens were estimated from 5-10% aliquots. The abomasum was ligated, incised along the greater curvature and the contents removed. The abomasum was washed and digested in pepsin/HCl according to procedures used by

Coop et al (1977).

Concurrently, portions of the small intestine and abomasum were taken for histological examination and were prepared according to procedures used by Coop et al (1976) and Coop et al (1977) respectively.

METHODS FOR DETERMINING ENDOGENOUS AND EXOGENOUS CALCIUM AND PHOSPHORUS IN DIGESTA AND FAECES

INTRODUCTION

The total Ca and P found in the abomasum, ileum and faeces is of exogenous and endogenous origin: the exogenous component comes from the food and the endogenous component originates either by diffusion from the blood or interstitial tissue fluid, as part of digestive secretions such as saliva, as a component of cells or cell fragments sloughed off from the intestinal lining, or contained in phagocytes.

The amount of endogenous Ca and P must be known to calculate the true absorption from the gut between the mouth and the site of sampling. This is particularly important for P as illustrated by the study of Lofgreen and Kleiber (1954) who showed that the apparent efficiency of absorption of P in alfalfa hay was 0.31 whereas the value for true absorption was 0.94.

To measure the endogenous component of digesta necessitates the use of radioactive Ca or P and to this end two methods have been developed: 1) the isotope dilution and 2) the comparative balance. The isotope dilution method was used

exclusively in these studies.

ISOTOPE DILUTION TECHNIQUE

The first methods for measuring endogenous Ca and P were based on the fact that the amount of radioactivity entering the gut is equal to that lost in the faeces. If it is assumed that the form of P which crosses biological membranes has the same specific activity (SR) as the SR in plasma, then $SR_p \times E_p = SR_F \times F_p$ where SR_p and SR_F represent the specific activity in plasma and faeces respectively, Ep the amount of P originating from the body and Fp the P excreted in the faeces.

The main justification for the assumption that the SR of the transport form in plasma and the mean SR of plasma are the same comes from the relationship between the SR in plasma and urine. Since the P in urine originates entirely from the P in plasma, any failure to achieve uniformity of labelling in the plasma would result in the SR of plasma and urine being different. There is, however, clear evidence for Ca and P, that the SR are the same (Wright, 1955b; Young et al, 1966c).

The first attempt to develop a practical method for measuring the endogenous faecal P was made by Kleiber et al (1951) who used repeated subcutaneous injections of ^{32}P to produce a constant SR in the plasma. They assumed that the amount of ^{32}P secreted into the intestinal contents during one day will, on ave rage, be excreted in the faeces two days later, thus

$$(SR_p \times E_p)_t = (SR_F \times F_p)_{t+2} \quad \text{or} \quad \frac{E_p}{F_p} = \frac{SR_F\ (t+2)}{SR_p\ (t)}$$

Lofgreen and Kleiber (1954) simplified this method by showing that it was sufficient to give a single subcutaneous injection at the start of the experiment and that there was a close correlation between the plasma and faecal SR after the fourth day. Moreover, the relationship between the SR of P in plasma and faeces with time was logarithmic from day four to seven and linear from day seven to 14. It was sufficient therefore, to use this linear part of the curve to give the proportion of the faecal P which is of endogenous origin. This method has been used as the standard method for calculating the endogenous P and Ca component of digesta and faeces.

Lofgreen (1960) determined the time lag required for a given level of activity in the plasma to be reflected in the faeces by showing that the peak in faecal SR occurred during the second day following injection. Therefore he argued that a given level of activity in the plasma can be assumed to be reflected in the faeces approximately two days later.

The method as developed by Lofgreen and Kleiber (1954) is a variant of a more general method which is based on the principal of radioisotope theory that the ratio of the area under the secondary curve (faeces) to that under the primary curve (plasma) gives the proportion of tracer(and tracee) irreversibly disposed of from the secondary pool that was derived from the primary pool (Shipley and Clark, 1972). Thus (E), the rate of flow of P or Ca to the gut (pool b) from plasma (pool a) is given by

$E = \frac{\text{Area b}}{\text{Area a}}$ x faecal P output or flow of P in digesta

In practice, little error is introduced by having a relatively early cut-off time.

It is simple to show that with the linear part of the curve the ratio of area and the ratio of the mean SR (Lofgreen and Kleiber, 1954) will give the same value for the fraction of faecal P of endogenous origin.

For a linear part of the curve the area is given by a trapezeoid of area

$$\tfrac{1}{2}\, t\, (SR_{(t+9)} + SR_{(t+2)}) \text{ for faeces and}$$

$$\tfrac{1}{2}\, t\, (SR_{(t+7)} + SR_{(t)}) \text{ for plasma, therefore}$$

$$\frac{A_F}{A_P} = \frac{\tfrac{1}{2}\, t\, (SR_{(t+9)} + SR_{(t+2)})}{\tfrac{1}{2}\, t\, (SR_{(t+7)} + SR_{(t)})} = \frac{\text{mean } SR_F}{\text{mean } SR_P}$$

The theoretical advantage of the area method is that first, all available data are used and secondly, and more importantly, this method is less sensitive to the delay between excretion of P into the gut and its subsequent excretion in the faeces.

EXPERIMENTAL PROCEDURE

Preparation and administration of ^{32}P and ^{45}Ca

The isotopes were supplied by the Radiochemical Centre, Amersham, England: ^{32}P (5 mCi/mg P) in the form of a sodium phosphate solution made isotonic with phosphate buffer and ^{45}Ca (25 mCi/mg Ca) as calcium chloride in aqueous solution. No additional carrier was added to the ^{32}P or ^{45}Ca which

were dissolved in 5 ml of 0.9% saline and administered intravenously as a single dose into the right jugular vein.

Sampling procedures

In the experiment carried out in the first series the method of Lofgreen and Kleiber (1954) was used with the following modifications: the dose of ^{32}P was given intravenously rather than intramuscularly and samples of plasma, faeces and urine were taken: every other day for plasma and every day for faeces and urine. Earlier studies had shown retention of the subcutaneous dose at the site of injection which, although without effect on the measurement of endogenous loss, would have interfered with any attempt to measure pool size and irreversible loss of P from the system.

There is no published method for measuring endogenous P utilising all the area under the SR - time curve for faeces and urine. The sampling procedure adopted for plasma was considered sufficiently comprehensive to obtain an accurate measure of the area. In the experiment carried out in the first series the blood sampling procedure was as follows: in the first 0.5 hour after dosing, samples were taken every 5 mins, in 0.5 - 1.5 h samples were taken every 10 mins, in 1.5 - 8.5 h samples were taken every 0.5 h, in 8.5 - 21.5 h samples were taken every hour and from 21.5 h onwards samples were taken twice a day.

Due to the frequency of blood sampling the animals were

fitted with bilateral jugular venous catheters and blood samples were taken from the left jugular vein using heparinised syringes. This was done to reduce the discomfort which would have been caused to the animal by numerous venous punctures.

As pool size was not measured in the second series of experiments the blood sampling programme was modified by taking fewer samples in the initial period after dosing and was as follows: in the first hour, samples were taken every 0.5 hour, in 2-6 h samples were taken every hour, in 6-14 h samples were taken every 2 h and from 24 h onwards samples were taken twice a day. Due to the reduced frequency of blood sampling the samples were taken from the left jugular vein by means of heparinised vacutainers (Becton - Dickinson, New Jersey).

For faeces the area under the curve is given by the formula $E_o^n SR_F t$, where n is the number of collections, SRF represents the SR of each collection of faeces and t the period of collection.

In the second series of experiments the fraction of Ca and P of endogenous origin in digesta was calculated using the area method.

Abomasal and ileal samples were taken 3, 6, 9 and 12 hours after dosing and then once daily.

Preparation of samples

Blood samples Blood samples were spun at 3100 g for 15

mins in an MSE Super Minor centrifuge (MSE Scientific Instruments, Crawley, England). The plasma was transferred by means of a glass pasteur pipette into 10 ml plastic tubes. The plasma was diluted immediately 1:5 with 10% (w/v) Trichloroacetic acid (TCA) and spun for 15 mins at 3100 g. The TCA precipitates the proteins and destroys the enzymic hydrolosis of the phosphoric esters to inorganic phosphate which occurs when blood is allowed to stand. The remainder of the plasma was stored at - 20 °C.

During the period of intensive bleeding the amount of plasma in the volume of TCA filtrate used to measure radioactivity was adjusted to take into account the rapid fall in activity in plasma which occurs within the first 24 h following dosing. The volume of TCA filtrate counted was 1 ml for samples collected in the first hour, 2 ml for samples taken between 1-2 h, 3 ml for samples taken between 2-3 h, 4 ml for samples taken between 3-4 h, 6 ml for samples taken between 4-6 h, 10 ml for samples taken between 6-8 h and 15 ml for samples collected from 8 h onwards. All samples were made up to 15 ml with water before counting.

Faeces samples Before ashing the faecal pellets were ground in a Moulinex grinder (Moulinex, Paris, France).

In the first series of experiments the faeces samples for counting of ^{32}P and ^{45}Ca were ashed as follows: concentrated nitric acid (16N) was added dropwise, with heating after each addition, to 1 g of wet faeces. This treatment was continued until the samples were pale yellow in colour.

Hydrogen peroxide (100 volume AR grade) was then added in small quantities until there was no further reduction of the pale yellow colour. The solution was then transferred to a 25 ml volumetric flask and made up to volume with distilled water.

In the second series of experiments faeces samples for counting of ^{32}P and ^{45}Ca were ashed in a Tecator Digestion System DS20 (Fack S - 26301 Höganäs Sweden). Two grams of wet faeces were weighed into Tecator tubes and 10 ml of sulphuric acid (36N) and two tablets of selenium catalyst (Kjeltabs ST 3.5 g K_2SO_4, 3.5 mg Se, Thompson and Capper Ltd., Runcorn, Cheshire) were added. Fifteen ml of hydrogen peroxide (100 vol AR) was added and a 10-15 min reaction time allowed before proceeding. The samples were put on the preheated heating block and left for 20 min at about 420 °C. The samples were removed from the heating block, allowed to cool for 10-15 mins and about 40 ml of water added. They were filtered through fibre glass paper (Whatman Glass Microfibre Paper 12.5 cm GF/D) washed with aliquots of water, transferred to 100 ml volumetric flasks and made up to volume.

Urine samples In the first series of experiments the urine samples (5 ml) were ashed according to the procedure for the faeces samples of this series.

Individual gut samples In the second series of experiments the individual unspun gut samples (about 3 g) were ashed according to the procedure for the faeces samples of this series.

Measurement of radioactivity due to ^{45}Ca and ^{32}P

^{45}Ca and ^{32}P are both β emitters and have a $\frac{1}{2}$ life of 165 and 14.2 days respectively. The radioactivity present in the samples was measured using a liquid scintillation spectrometer without automatic quench compensation (Nuclear Enterprises Ltd., Edinburgh, UK Model 8312).

In theory this type of equipment offers a number of possibilities for determining the radioactivity due to ^{45}Ca and ^{32}P present in the same sample. There are two basic approaches depending on whether or not chemical separation of the radionuclides is employed. Without chemical separation individual radionuclides can be determined by:-

a) measurement of total activity in a liquid scintillator before and after decay of ^{32}P. This would necessitate a delay of approximately 14 weeks between the two determinations to allow for decay of ^{32}P to approximately 1% of its original activity.

b) measurements of total activity in a liquid scintillator and an additional determination of the ^{32}P in a separate sample by Cerenkov counting and subsequent correction.

c) simultaneous differential counting in a liquid scintillator using the difference in the energies of the radionuclides to effect a separation. The extensive range of quench expected in samples of faeces, urine and plasma would be a particular problem with this approach and in the absence of automatic quench compensation would be a potential source of significant error.

In all these options the need to incorporate samples, especially faeces, into a liquid scintillator in a form which would allow a reasonable counting efficiency meant that some chemical manipulation would be inevitable in order to reduce colour quenching, and that this process might also increase chemical quenching. Because of these practical limitations it was decided at the outset to separate the ^{45}Ca and ^{32}P before counting. This had the advantage that an almost colourless solution would be produced which would allow the ^{32}P to be counted with high efficiency by Cerenkov counting and would minimise colour quench problems for the determination of ^{45}Ca by liquid scintillation counting. Chemical separation was also a necessary preliminary to the determination of stable Ca and P.

Determination of radioactivity due to ^{45}Ca by liquid scintillation counting

It was decided to separate the ^{45}Ca by precipitation as Ca oxalate; and it was necessary therefore to carry out preliminary experiments to select a suitable scintillator which would allow incorporation of an adequate proportion of aqueous acid solution of Ca oxalate of suitable concentration and provide reproducible counts. Of several liquid scintillators examined only two were capable of incorporating 10% of aqueous samples which was considered to be a minimum requirement. These were (a) Toluene/Triton - X - 100 2:1 containing 5 g 2, 5 - diphenyloxazole (PPO) and 0.3 g 2 - (5 phenyloxazolyl) benzene (POPOP) per litre of toluene, and (b) Minisolve (Koch Light, Colnbrook,

Bucks, UK).

Samples containing 0.5 ml of hydrochloric acid (HCl) in strength from 0.2 to 4 N and a standard quantity of ^{45}Ca were added to 4.5 ml of these two scintillators and counted daily for three days. From the spectrum for ^{45}Ca shown in Fig. 2.1 the channel setting used was from 1.0 to 6.0 volts. As can be seen from Figs. 2.2 and 2.3 the HCl acted as a quenching agent producing a linear quench curve which could be used for quench correction. However, although the samples in Toluene/Triton - X - 100 gave reproducible counts over three days those in Minisolve did not. This phenomenon illustrates unstable counting performance which is one of the potential hazards inherent in the use of emulsion type liquid scintillators and which has been fully described by Fox (1974a; 1974b). Since Toluene/Triton - X - 100 gave reproducible performance it was selected as the scintillator for the further development work.

To further establish the suitability of Toluene/Triton - X - 100 for this study it was necessary to establish whether a sufficient weight of Ca oxalate could be incorporated into 4.5 ml of scintillator. Table 2.1 shows the effect of the strength of HCl, used to dissolve the Ca oxalate precipitate, on the ability of this scintillator to incorporate Ca oxalate. Increasing the acidity increased the amount of oxalate that could be dissolved but also increased the degree of quench (Fig. 2.3). Since 3N HCl appeared capable of incorporating the maximum weight of Ca oxalate expected to occur in the experimental samples

this was selected as the concentration of acid to be used. The final scintillation cocktail therefore consisted of 0.5 ml of 3N HCl containing dissolved Ca oxalate and 4.5 ml of Toluene/Triton - X - 100 scintillator.

In addition to unstable counting behaviour already referred to, another problem which can arise in the use of emulsion type scintillators is due to their micellar nature. This results in uneven distribution of materials throughout the scintillator mixture and concentrates the fluors in the hydrophobic part of the micelle. Conversely the water-soluble components are located in the hydrophilic regions and if the radioactive material in the sample is hydrophilic it will reside in a part of the micelle remote from the scintillator. With low energy β emissions this may significantly reduce the counting efficiency due to quenching within the micelle, and estimation of the degree of quench using an external γ source, which will have much greater effective range through the solution, may give a value which does not reflect the degree of quenching experienced by the β label. The problem increases with the polarity of the labelled compound and with a decrease in energy of the β particle. In practice with low energy β emitters it means that if an external standard quench correction technique is to be used it must be shown to be valid. Alternatively if quench correction is required a sample channels ratio technique must be used. For ^{45}Ca which has a β max of 0.25 MeV the problem does not exist and consequently because of its inherent advantages an

external standardisation technique was used to construct the quench curves shown in Fig. 2.2 and 2.3.

The regression data was stored in a computer (PDP - 8f, Digital Equipment Corporation, Maynard, Massachusetts) which was used for calculation of the results.

Preparation of calcium oxalate

(a) Faeces and digesta

One ml of ashed faeces or digesta in a conical centrifuge tube was treated with two drops of bromophenol blue indicator and 1 ml of saturated ammonium oxalate solution. Sodium hydroxide (0.5 N) was added until the solution turned blue. After 30 mins the mixture was centrifuged for 15 mins at 3100 g and the supernatant carefully decanted. The precipitate was washed with 4 ml of dilute ammonia, recentrifuged, dissolved in 1 ml of 3 N HCl and 0.5 ml added to 4.5 ml of scintillator. Blanks consisting of HCl and scintillator, and standards containing 0.01 μCi ^{45}Ca were included with every set of samples. The output from the counter was recorded on punch tape and the tape fed directly into the computer. Results were expressed as % standard.

(b) Plasma

Ca from 2 ml of plasma was precipitated using 2 ml ammonium oxalate and then treated as above.

Determination of radioactivity due to ^{32}P by Cerenkov counting

Samples which have been subjected to chemical treatment are

particularly suitable for Cerenkov counting since chemical quenching does not occur in this process. Consequently restrictions on chemical manipulation are minimal and so decolourisation procedures can be used to minimise colour quenching which does occur. Other advantages of this technique include elimination of liquid scintillator which reduces cost and allows the sample to be recovered for subsequent analysis and the ability to use larger sample volumes, which more than compensates for the lower counting efficiency. The technique is limited, however, to β emitters of high energy since Cerenkov radiation is only emitted above an energy threshold which can produce β particles capable of travelling with a velocity greater than light in a particular medium. For this reason the energy threshold varies with the refractive index of the medium and for water is 0.263 MeV. Consequently this technique is not applicable to the measurement of ^{45}Ca.

Cerenkov radiation differs from the light emitted by liquid scintillators in two respects which are of practical significance. First, Cerenkov radiation is of lower wavelength, (in the near ultra voilet), which is less well matched to the photo peak sensitivity of the photomultiplier tubes used in liquid scintillation counters and thus results in a decreased counting efficiency. Because of this, wavelength shifters have often been used in order to match the light output more closely to the photomultiplier tubes, but these compounds place limitations on the chemical treatment of the sample (Smith, 1978) and prevent recovery of uncontaminated sample. For these reasons

wavelength shifters were not used.

The short wavelength of Cerenkov radiation also means that yellowish colouration of the sample solution is particularly effective in absorbing the light and can thus cause serious colour quenching. A second characteristic of Cerenkov radiation is that it is directional in nature and this also contributes to a lower counting efficiency in coincidence type counters since some of the light may only be detected by one photomultiplier tube. However in spite of these limitations counting efficiencies in aqueous media of the order of 40% for ^{32}P can be achieved in practice.

Quench correction As already mentioned chemical quenching does not occur in Cerenkov counting. Colour quenching can, however, be a problem and quench correction may be necessary. Variation between vials may also be a factor contributing to variable quench (Parker, 1974; Smith, 1981).

In this study it was found necessary to apply a quench correction for ashed samples of faeces and urine. The external standard often cannot be used for quench correction in Cerenkov counting since the gamma source provided in most machines is unsuitable for producing Cerenkov radiation. The liquid scintillation counter used in the present work fell into this category. It was therefore necessary to use a sample channels ratio technique which, however, suffers from the disadvantage that longer counting times are necessary to allow the accumulation of sufficient counts in each channel to provide a precise estimate of the ratio.

From the spectrum for ^{32}P shown in Fig. 2.4 the channel settings which provided a suitable channels ratio and detection efficiency were from 0.3 to 1.1 and from 0.3 to 4.0 volts. This technique was found to be capable of correcting for colour quench as can be seen from the quench curves shown in Fig. 2.5. It is also apparent from Fig. 2.5 that separate quench curves were necessary for urine and faeces. This latter fact emphasises the importance of preparing quench curves using the same quenching agent which occurs in the experimental samples. Plasma TCA filtrates were colourless and quench correction was unnecessary.

The regression data for each sample type were stored in a computer programme which was used for calculation of the results.

Counting of samples Fifteen ml of the ashed solution of faeces, urine or digesta and 15 ml of plasma TCA filtrate were pipetted into vials. Blanks consisting of 15 ml of water and standards containing 0.1μCi ^{32}P (nominal) were included with each set of samples. The output from the counter was recorded on punch tape and the tape fed directly into the computer. Results were expressed as % standard.

MEASUREMENT OF THE FLOW OF DIGESTA ALONG THE GASTRO-INTESTINAL TRACT

INTRODUCTION

In studies of digestion it is often necessary to measure the amounts of nutrients that enter and leave the various sections of the alimentary tract. With ruminants a convenient technique for making such measurements involves the use of

markers which travel with the digesta or with some fraction of the digesta.

The flow of digesta past a given point is equal to

$$\frac{\text{the daily intake of marker}}{\text{concentration of marker in the sample}}$$

and the rate of flow of a component of the digesta past a given point is equal to the flow of digesta x the concentration of component in the digesta collected through a permanent cannula established at that point in the tract.

THEORY

For a substance to qualify as a marker in nutritional studies, it should: be inert with no toxic, physiological or psychological effects; be neither absorbed nor metabolised within the alimentary tract; have no appreciable bulk; mix intimately with the usual food and remain uniformly distributed in the digesta; have no influence on alimentary secretion, digestion, absorption, normal motility of the digestive tract or excretion; have no influence on the microflora of the alimentary tract which is of significance to the host; have qualities that allow ready precise quantitative measurements; have physical - chemical properties which make it discernible throughout the digestive process.

The markers used in ruminant nutrition can be divided into two categories, liquid phase markers such as polyethylene gylcol (PEG) and ^{51}Cr EDTA, and solid phase markers such as lignin, ^{144}Ce and ^{103}Ru(phen).

The most widely used water soluble marker is PEG. However, a serious limitation in the use of PEG is the lack of a specific, sensitive and accurate method of analysis. Downes and M cDonald (1964) showed that ^{51}Cr EDTA is a satisfactory soluble marker in spite of the slight absorption and subsequent excretion in the urine, the maximum amount excreted being 4.7% of the dose.

Similar problems exist with the use of lignin as a solid phase marker. The assay is laborious, and the lignin may be absent from some diets, or in other diets digested in significant amounts in the alimentary tract. Tan et al (1971) showed that (^{103}Ru(phen)$_3$) Cl_2 was not absorbed to any significant extent in the alimentary tract and was found to be adsorbed on solids of digesta therefore making it a suitable solid phase marker.

In this study ^{51}Cr EDTA was used as the marker in the liquid phase and (^{103}Ru(phen)$_3$) Cl_2 as the marker in the particulate material. Radioactivity was selected as a means for sensitive and completely specific analysis.

The markers can be used in conjunction with T - piece or re-entrant cannulae. With re-entrant cannulae the flow rate can be estimated directly and the marker is used to correct the estimated digesta flow rates to 100% recovery of marker.

In this study T - piece cannulae were used, the markers administered as a continuous intraruminal infusion and samples of digesta collected when equilibrium was achieved - i.e. marker concentration at any sampling point will be

constant.

A problem associated with the use of T - piece cannulae is the difficulty of collecting samples that contain both particulate material and dissolved substances in the same proportions as exist in the digesta flowing past the cannula. The sampling difficulty can be overcome by using a combination of solid phase and liquid phase markers. However the flow of each phase can only be accurately estimated if the markers associate exclusively with and are distributed uniformly throughout one or other phase, a difficulty that can be avoided if the true composition of digesta passing the sampling point is determined from the marker concentrations.

Faichney (1975) has developed a method for measuring the true composition of digesta, which does not require that each marker associates exclusively with one phase. This method is based on the principal that, given that equilibrium has been achieved and is maintained by a continuous infusion of the two markers, the concentration of those markers (expressed as a fraction of the daily dose per unit of digesta) in digesta flowing past any sampling point must be equal. If a sub sample of fluid is prepared by straining or centrifugation of a sample of digesta so that fluid and digesta contain different proportions of liquid and particulate matter, the true digesta can be reconstituted by combining fluid and digesta, either mathematically or physically, so that the concentrations of the two markers are the same.

If x = a quantity of digesta (D)

y = a quantity of fluid (F) which, when added to or

removed from x, reconstitutes true digesta (TD).

S_D, S_F, S_{TD} = concentrations of the solute marker (^{51}Cr EDTA)

P_D, P_F, P_{TD} = concentrations of the particle marker (^{103}Ru(phen))

then $x\ S_D + y\ S_F = x\ P_D + y P_F$

so that $\frac{y}{x} = \frac{P_D - S_D}{S_F - P_F} = R$ (1)

where R is the reconstitution factor i.e. the number of units of fluid that must be added to (or removed from) one unit of digesta to obtain true digesta.

Then $\frac{S_D + R\ S_F}{1+R} = S_{TD} = \frac{P_D + RP_F}{1+R} = P_{TD}$ (2)

and flow of TD = $1/S_{TD} = 1/P_{TD}$ (3)

The flow of any constituent of true digesta eg organic matter, dry matter, Ca and P can be calculated by substituting its concentration for that of the marker in equation (2) and multiplying by the flow calculated in equation (3).

eg $\frac{Ca_D + R\ Ca_F}{1+R}$ x Total flow = flow of Ca in total digesta (1)

Flow of liquid x Ca_F = flow of Ca in liquid phase (2)

Subtract (1) - (2) = flow of Ca in solid phase

Abomasal and ileal total flows were calculated using this method.

EXPERIMENTAL PROCEDURE

Preparation and administration of ^{51}Cr and ^{103}Ru

^{103}Ru Cl_3 (SR 1.5 mCi/mg Ru) and ^{51}Cr EDTA (SR 1.5 mCi/mg Cr) were supplied by the Radiochemical Centre, Amersham, UK. Before administration to the animal, the ^{103}Ru Cl_3 was

converted to tris (1, 10 - phenanthroline) ruthenium (II) chloride by the method of Tan et al (1971) as follows: - carrier $RuCl_3$ in 2N HCl was added to reduce the SR to 1 μCi/mg Ru. Potassium pentachlorohydroxyruthenate was formed by refluxing 0.5 g $RuCl_3$ with 0.20 g KCl, 40 ml ethanol (absolute) and 35 ml 0.2 N HCl for 20 mins, followed by removal of the ethanol by distillation. After cooling, 0.7 g 1, 10 - phenanthroline and 0.175 g sodium hypophosphite were added to the residue in the flask and the contents refluxed for four hours to form $^{103}Ru(phen)_3Cl_2$. The solution was filtered, made to 50 ml and the yield determined by counting samples of the complex and original solution.

The SR of the ^{51}Cr EDTA was also reduced by addition of non-radioactive Cr EDTA, prepared as described by Binnerts et al (1968), to give a final SR in the infusate of 1 μCi/mg Cr.

Both markers were incorporated into a single batch of solution sufficient for each experiment. The dose was infused into the rumen using a peristaltic pump (Watson and Marlow, Falmouth, UK. Model MHRE 2) set to deliver 70 ml/day from a separate plastic container for each sheep. The dose rate was checked by weighing each container daily.

Sampling procedures

Abomasal and ileal samples were collected by attaching a plastic bottle to the sampling port of the cannulae. Ruminal samples were aspirated through the fistula using a polythene tube attached to a 50 ml syringe. Samples were taken at 0.900, 13.00 and 17.00 h on the last two days of the collection period, pooled on an equal weight basis and stored at 4 ^{o}C.

Aliquots of the pooled abomasal and ileal samples were centrifuged at 50,000 g for 30 mins in a HiSpin 21 centrifuge (MSE Scientific Instruments, Crawley, UK) and the supernatants stored at 4°C. The pellets were discarded.

Due to the difficulty in obtaining a representative sample of the ruminal contents, whole samples were centrifuged as above and the liquid and solid phases stored separately, at 4°C and after freeze drying respectively.

Measurement of radioactivity due to ^{51}Cr and ^{103}Ru

Both ^{103}Ru and ^{51}Cr are γ emitters and have half lives of 40 and 27.8 days respectively. The radioactivity present in the samples was measured on a Nuclear Enterprises 8311 spectrometer fitted with a 3" sodium iodide crystal.

Since the similarity of the half lives would not permit measurement after differential decay it was necessary to measure the activities by simultaneous counting, and by separating the two radionuclides on the basis of their energy spectra. As can be seen from Fig. 2.6 the spectra are not discrete, but by careful selection of energy levels it was possible to reduce the overlap to a satisfactory level. The final settings used were 3.9 to 4.1 volts for ^{51}Cr and 4.2 to 4.8 volts for ^{103}Ru, which produced a count rate due to ^{51}Cr in the ^{103}Ru channel equivalent to 0.3% of that in its own channel. Conversely the contribution from ^{103}Ru to the ^{51}Cr channel was equivalent to 11% of the count rate in its own channel. In order to compensate for any drift in the machine performance which might affect the extent of these overlaps, separate ^{51}Cr and ^{103}Ru standards were

included at the beginning of each set of samples to enable the exact degree of overlap to be calculated.

In order to obviate the need for geometrical correction for variation in sample volumes all samples were adjusted to 10 ml volume; 2 ml of the supernatants from the pooled ruminal, abomasal and ileal samples diluted to 10 ml with distilled water, 10 g of the pooled abomasal and ileal contents before centrifugation and a weighed amount (0.5-1.0 g) of the freeze dried ruminal solid phase samples sufficient to fill the counting vial to the 10 ml mark were taken for counting. Water blanks and mixed standards containing 0.5 ml infusate (0.3572 μCi ^{51}Cr and 0.0715 μCi ^{103}Ru) were included with each set of samples. Results were calculated as % mixed standard using the computer.

BALANCE TRIALS

INTRODUCTION

In these experiments surgically modified sheep equipped with cannulae, were used to study the movement of endogenous and exogenous P and Ca in lambs parasitised with *O. circumcincta* and *T. colubriformis*.

EXPERIMENTAL ANIMALS AND DIETS

The animals used in these experiments were selected from a large groups of cross bred (dam, Blackface X Border Leicester; sire, Suffolk) lambs which had been reared indoors free of parasites from birth.

Before the start of the experiments the selected animals were fitted with ruminal, abomasal and ileal T - piece cannulae. In the second series of experiments the abomasal

cannulae were placed between the ribs in the last intercostal space and the ileal cannulae were placed higher up the ileum than in the first series of experiments.

At all times the animals were fed the complete diet Ruminant A (Appendix 2.1). A detailed description of its composition and analysis was given by Wainman, et al (1970). were

Management of experimental animals

During the balance trials the sheep were housed in single metabolism crates fitted with automatic feeders and glass fibre separators. The separators were fitted with nylon sieves for the separation of faeces and urine. When the animals were not taking part in a balance trial they were housed in either double metabolism crates or holding pens.

The surgical modifications were inspected daily, cleaned and the surrounding wool trimmed as required.

EXPERIMENTAL PROCEDURE

Collection of samples

Diet samples Before each balance trial enough bags of diet to last the whole trial period were weighed out and sampled at intervals to form a running sample for moisture, Ca and P determinations. The trial period was divided into a preliminary and a collection period. The animals were accustomed to metabolism crates and automatic feeders.

Food refusals were collected, weighed and recorded daily when the animals were being dosed with larvae.

Faeces and urine samples Each day of the collection period the separators were brushed down and sprayed with 300 ml

of 5% acetic acid. The urine and faeces buckets were changed every day and the outputs recorded. One hundred ml of 50% acetic acid was added to each clean urine bucket to acidify the urine in order to prevent the precipitation of P as phosphates.

A 10% sample of faeces or urine was taken for each sheep and bulked over the collection period to form a composite sample.

Samples of digesta Ruminal, abomasal and ileal samples were taken at four hourly intervals i.e. 0.900, 13.00 and 17.00 on the last two days of the collection period and pooled on an equal weight basis to form a composite sample. These samples were spun as previously described for flow measurements.

Preparation of samples for analysis

At the end of the collection period a figure for moisture content was obtained for samples of ground diet, 10% faeces, unspun abomasal and ileal composites and spun ruminal, abomasal and ileal composites. The ground diet or 10% faeces samples were weighed into an aluminium tray and dried to a constant weight at 100 ^{o}C in a force draught oven. The spun or unspun composite digesta samples were weighed into a silica crucible and evaporated to dryness on a hot-plate. The tray or crucible and dried sample were reweighed and a figure for moisture content calculated.

The dried faeces were ground in an Osteriser Dual Range Pulse Matic 16 (Oster Corporation, Milwaukee, Wisconsin 53217) before dry ashing. The grinder was placed in a fume

cupboard and covered with a plastic bag to ensure that there was no escape of radioactive dust.

Two to 3 g of the dried ground diet, 2 g of dried ground faeces, 15 ml of the 10% urine (evaporated to dryness), 10 g of the unspun abomasal and ileal, 5 ml of the spun ruminal, abomasal and ileal or 1 g of the freeze dried rumen solid phase composite samples were placed in a weighed silica crucible and ashed in a muffle furnace at 600 ^{o}C for 12 hours. After ashing, 10 ml of 50% HCl was added and the solution heated on a hot-plate for 30 mins, filtered into a 100 ml volumetric flask and made up to volume.

In the second series of experiments a figure for organic matter was calculated for diet, faeces, spun and unspun abomasal and ileal samples after dry ashing.

METHODS OF ANALYSIS

In all experiments of series one and two the concentrations of P and Ca were determined in the plasma and ashed solutions. In series one plasma concentrations of pepsinogen were also determined. In series two additional determination of the weekly changes in plasma concentration of pepsinogen, total protein, albumin and globulin were made.

AUTOMATED METHODS

Chemical analyses were carried out using automated flow analysis, batch analysis, atomic absorption spectrometry and emission flame photometry.

Automated flow analysis

Flow lines Flow lines consist of a number of modules (such

as mixing coils, dialysers, heating coils, incubation coils and colorimeters) linked together by a network of glass tubing through which reagents are pumped continuously by a peristaltic pump. A sampler module presents samples in turn to a sampling probe which measures the required amount of material to be injected into the system. Samples follow each other successively along the tubing, and carry over between samples is reduced by introducing a regular pattern of air bubbles into the liquid stream. The bubbles prevent the samples from diffusing into each other. There is an interval after each sample has been introduced into the system during which a saline wash flows through the sampling probe. Sampler timings are 30 sec sampling: 60 sec wash. The following assays were carried out using automated flow lines.

Inorganic phosphate

Plasma P concentrations were measured using the method of Robinson et al (1971). Plasma is diluted with and dialysed into 1% sulphuric acid. The inorganic phosphate in the dialysate is coupled with a molybdivanadate reagent, and the intensity of the yellow complex read at 403 nm. Two reagents are required: a diluent of 1% sulphuric acid (requiring the addition (1 ml/l) of octan - 2 - ol as a wetting reagent) and a colour reagent (a mixture of solutions of ammonium metavanadate and ammonium molybdate in 50% nitric acid). Aqueous solutions 2, 4, 6, 8, 10 mg/100 ml of potassium orthophosphate (BDH product No 10203) were used as standards.

Calcium

Plasma Ca concentrations were measured using an Auto

Technicon method, N - 31P. A fluorescent complex is produced when Ca is added to a strongly alkaline solution of calcein. Magnesium does not interfere under these conditions. The complex is activated at 405 nm and the fluorescence above 405 nm is measured. The fluorescence maximum occurs around 520 nm.

Plasma samples were initially diluted with 0.9% saline and then mixed with an alkaline solution of calcein. The mixture then passed to the fluorometer where it was activated and the fluorescence measured. Two reagents were required: 0.9% saline for dilution of the plasma samples and an alkaline solution of calcein (calcein in 50% sodium hydroxide). Aqueous solutions of Ca carbonate (BDH product No 10068), 5.0, 7.5, 10.0, 12.5, 15.0 mg/100 ml were used as standards.

Total protein

Plasma total protein concentrations were estimated using the biuret method described by Weichselbaum (1946). In the presence of a tartrate diluent, copper in alkaline solution reacts with the peptide bonds of proteins to give a deep purple complex. The developed colour was read at 550 nm. Only one reagent (containing tartrate, copper sulphate, potassium iodide and sodium hydroxide) was used in the assay. Aqueous solutions, 1, 2, 4, 6, 8 g/l of bovine albumin fraction V (BDH product No 44155) were used as standards.

Albumin

Plasma albumin concentrations were determined using the method described by Ness *et al* (1965). This is based on the quantitative binding of the anionic dye, 2 - (4 -

hydroxyazobenzene) - benzoic acid (HABA), with albumin. Colour intensity was measured at 499 nm. Albumin blanks were determined in a flow line identical to that used for albumin determination, except that HABA was replaced by phosphate buffer. The same standards as those used in total protein estimations were used in both albumin and albumin blank determinations.

Globulin

Plasma globulin concentrations were taken as the difference between total protein and albumin.

Batch analysis

Sample preparation unit The sample preparation unit (Hook and Tucker Instruments Ltd., Croydon, England) consists of a rotating mechanism containing concentric rings of sample and reaction tubes and two sampling probes. One probe picks up the sample from the inner ring and transfers the sample, diluted with reagent, to the outer ring of reaction tubes. The second probe transfers part of the contents of the reaction tube to the flow cell in a colorimeter. Reaction time in the unit was constant, but the volumes of sample and reagent variable.

The following assay was carried out using the sample preparation unit.

Inorganic phosphate

The P concentrations of plasma and ashed samples were determined using the method of Robinson et al (1971) as previously described but without the diluent and dilution of the colour reagent 1:5 with water before use. As the plasma

P concentrations were determined on the TCA filtrates, the standards were made up in TCA. For ashed faeces and digesta samples, aqueous solutions, 25, 50, 100, 150, 200 μg/ml and for ashed urine samples, aqueous solutions, 10, 20, 30, 40, 50 μg/ml of potassium ortho phosphate (BDH product No 10203) were used as standards.

Atomic absorption

The atomic absorption spectrometer measures the concentration of metallic elements in a variety of materials. In atomic absorption analysis, a sample, usually in solution is converted into an atomic vapour by an atomiser, most commonly a flame, and irradiated by the light from a source. This source must emit radiation that is specific to the element being analysed. The absorption of this radiation by the atomic vapour is measured.

The following assay was carried out using atomic absorption.

Calcium

When the samples were prepared for ^{45}Ca counting the remaining oxalate precipitate, dissolved in 3N HCl, was used for Ca determination by this method. The Ca concentrations of plasma and ashed solutions were estimated by atomic absorption using a Varian Techtron 1100 (Varian Ltd., Walton-on-Thames, England). Samples were diluted (1:25v/v) with an aqueous solution of lanthanum chloride (2.66% w/v $LaCl_3 . 7H_2O$) (BDH product No 14041). Standards used consisted of aqueous solutions, 80, 90, 100, 110, 120 mg/ml of calcium nitrate (BDH product No 14136).

A comparison was made between the fluorometric method used

in series one and atomic absorption in series two. Ca concentration was measured in 22 plasma samples by the two methods but no significant difference was found between methods

Emission flame photometry

Any substance, when exposed to sufficiently high temperatures, will be forced into an excited state through thermal collision. Since these states are unstable, the excited atoms or molecules will return to the ground state, dissipating the absorbed energy in various ways, one of which is light emission. Each atom or molecule has associated with it a set of discrete energy levels. In the separated, i.e. atomised state, therefore, excited atoms will emit a characteristic set of wavelengths termed a "spectrum". The intensity of the light so emitted is directly proportional to the number of atoms undergoing the transition. Thus, by selectively monitoring a characteristic wavelength of an element being volatised and excited in a flame, the concentration of that element may be measured directly.

The following assay was carried out using flame photometry.

Sodium

The sodium concentrations of the ashed digesta samples were estimated by flame photometry using an IL 343 Digital Flame Photometer (Instruments Laboratory Inc., Lexington, Massachusetts, USA).

The concentration of sodium was determined in an appropriate dilution of the samples with a solution of lithium nitrate (15 m Eq/ℓ) (BDH product No 29076). Solutions of sodium

chloride (BDH product No 22054) (0.001, 0.0025, 0.005 g/100 ml) diluted with lithium nitrate were used as standards.

Calculation of results

Absorbances of standards and unknowns for both the flow line and sample preparation unit methods were recorded as peaks on a moving chart recorder attached to the colorimeter. The atomic absorption equipment was also linked to a moving chart recorder. Peak heights were measured using a pencil follower (d - mac Ltd., Glasgow, Scotland) which determined the X and Y co-ordinates of the peaks and displayed them on a digital display module. The digital output of the pencil follower was recorded on punch tape and the tape fed directly into a PDP - 8f computer.

MANUAL ASSAYS

Calcium

The Ca concentrations of ashed samples were determined using the method of King and Wootton (1956). One ml of ashed sample in a conical centrifuge tube was treated with two drops of bromophenol blue indicator and 1 ml of saturated ammonium oxalate solution. Sodium hydroxide (0.5 N) was added until the solution turned blue. After 30 mins the mixture was centrifuged for 15 mins at 3100 g and the supernatant fluid poured off carefully. The precipitate was washed with 4 ml of dilute ammonia and recentrifuged. The washed precipitate of calcium oxalate was dissolved in 2 ml of N sulphuric acid by warming the tube to 70-80 ^{o}C in a beaker of water. Titration was carried out by adding, dropwise, 0.01 N potassium permanganate (BDH product No 18017). The

tube was kept warm by immersion in the hot water during titration. A blank titration was carried out with 2 ml of N sulphuric acid. The amount of permanganate used in the blank was subtracted from the amount used for the samples.

The amount of Ca in the samples (g/100 g) was calculated from 1 ml N/100 $KMnO_4$ = 0.0002 g Ca.

Pepsinogen

The pepsinogen was analysed by a radial diffusion method. Two grams of Agarose was dissolved in 100 ml of boiling Tris buffer. The Agarose was added to cold buffer and heated until boiling. After all the Agarose had dissolved, the solution was placed in a water bath at between 50-60 °C, 50 ml of casein solution added (0.5 g in 100 ml) and the mixture used to pour the plates. The plates were poured on a level surface ensuring an even layer of gel across the plate. After the gel had set the plates were immersed in Glycine - Barium buffer for two hours. After removal from the buffer, holes (approximately 1.5 cm apart) were punched in the gel using the template and a gel cutter with 0.4 mm diameter.

A series of standards were made up using pepsin (Sigma product No P-7012) in the range of 1 mg/ml to 0.0001 mg/ml in ten-fold dilutions using Tris buffer. Ten µl of sample or standard was used per well. Best results were obtained by adding a further 10 µl to the well after the first application had soaked into the gel.

The plates were placed in a moist chamber (sandwich box) and incubated at 37 °C overnight. Diameters of clear areas in

the opaque gel were measured and the results recorded. A standard curve was plotted from diameters of standards using log concentration of pepsinogen v diameter of clear zone. Unknown volumes were read off from the standard curve as ng/ml pepsin.

However, as the majority of work on parasitism in ruminants quotes pepsinogen values as milli Units of tyrosine per litre of sample per minute (after the method of Anson and Mirsky 1932), the values for pepsinogen concentrations have been converted into these units for ease of comparison.

ANALYTICAL QUALITY CONTROL

Quality control in automated assays consisted of checks performed by the computer on various criteria: an example of the computer print-out is given in Appendix 2.2. The criteria used included the regression coefficient and residual variance of the best line through the standards, and also the mean difference between duplicates. Samples were analysed in batches (usually of 64 samples); if the difference between individual duplicates within batches, exceeded three times the median duplicate difference (=SD) then the results for that sample were rejected.

Inorganic standard curves were corrected against versatols (pooled human serum, standardised for several constituents: William R Warner, Eastleigh, Hants), and the factor by which the estimated standards were multiplied as a result of the versatol correction was printed out. Versatols were not used in protein assays; the N content of the bovine albumin used as the standard was used as a correction

factor.

This use of correction factors was considered to provide adequate quality control between batches. Within batches, quality control was monitored by assaying pooled ovine plasma samples, one after every 10 samples. Machine drift within runs was thus monitored by the changes in pool plasma concentrations, and also by the changes in initial and final base line and Versatol levels. The order of samples within a typical run was five standards, blank, versatol, four unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, blank, Versatol.

TABLE 2.1 The effect of the strength of HCl (0.5 ml) on the dissolution of Ca oxalate

mg Ca oxalate	0.2N HCl	0.3N HCl	0.4N HCl	0.5N HCl	1N HCl	2N HCl	3N HCl	4N HCl
	0.5 mg*	0.5 mg*	0.5 mg*	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg
		1 mg*	1 mg*	1 mg	1 mg	1 mg	1 mg	1 mg
				2 mg	2 mg	2 mg	2 mg	2 mg
						4 mg*	4 mg	4 mg
						6 mg*	6 mg	6 mg
							8 mg*	8 mg
							10 mg*	10 mg*
							11 mg*	11 mg*
							12 mg*	12 mg*
								13 mg*
								14 mg*
								15 mg*

* Heated

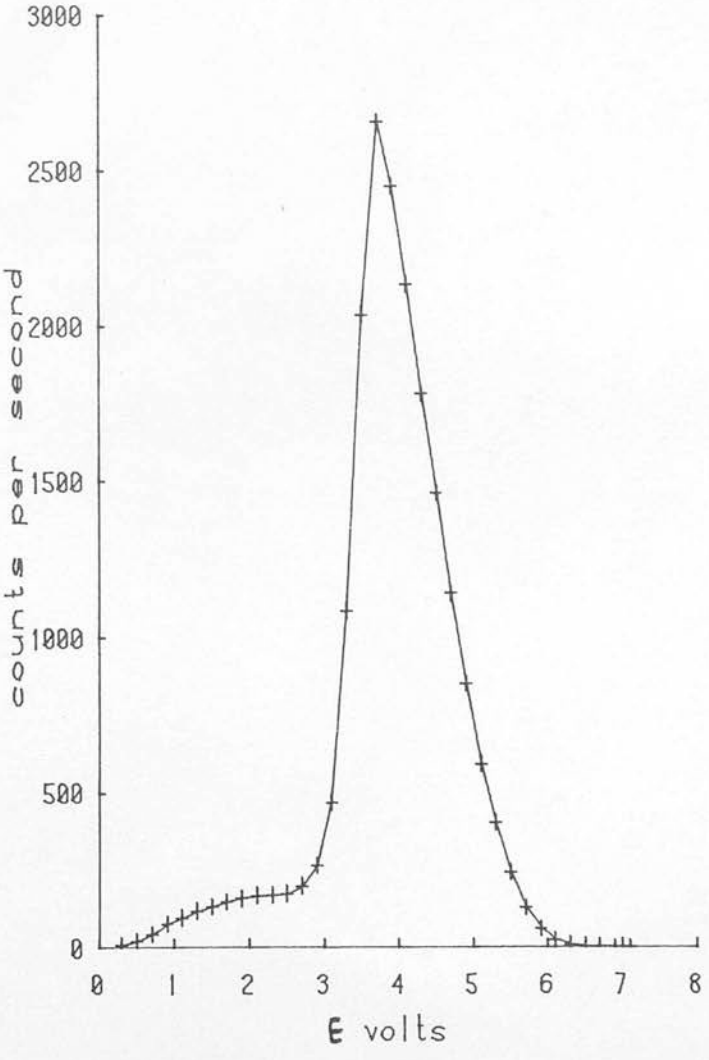

Figure 2.1 Spectrum for Ca-45

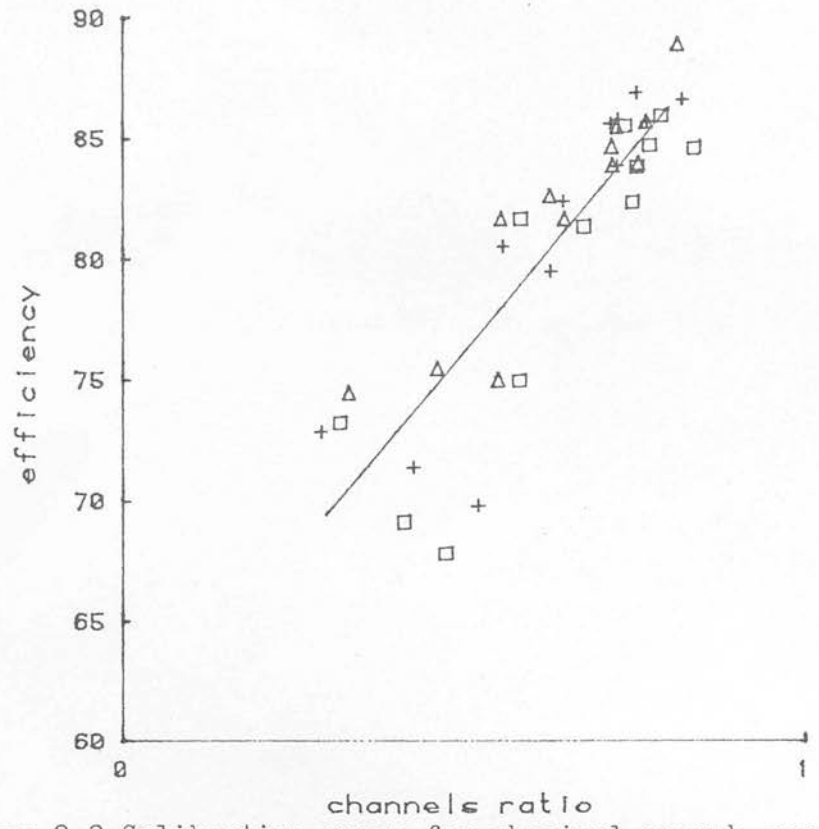

Figure 2.2 Calibration curve for chemical quench correction in counting Ca-45 in Minisolve using different strengths of HCl as quenching agent.
△ 1st count + 2nd count □ 3rd count

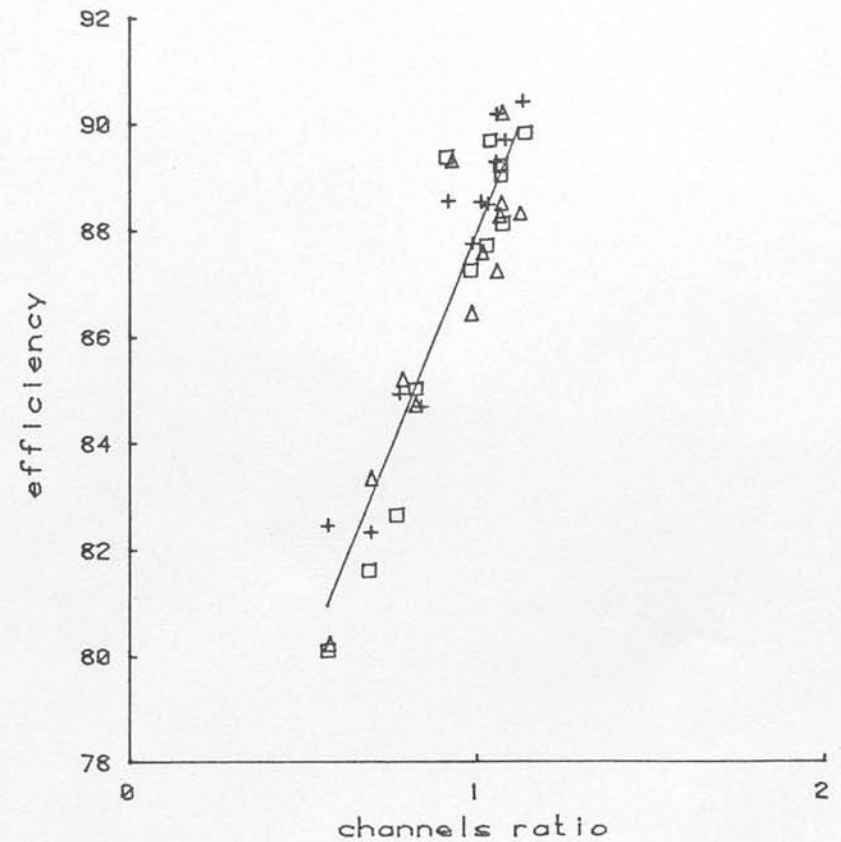

Figure 2.3 Calibration curve for chemical quench correction in counting Ca-45 in Toluene/Triton-X-100/PPO/POPOP using different strengths of HCl as quenching agent.
△ 1st count + 2nd count □ 3rd count

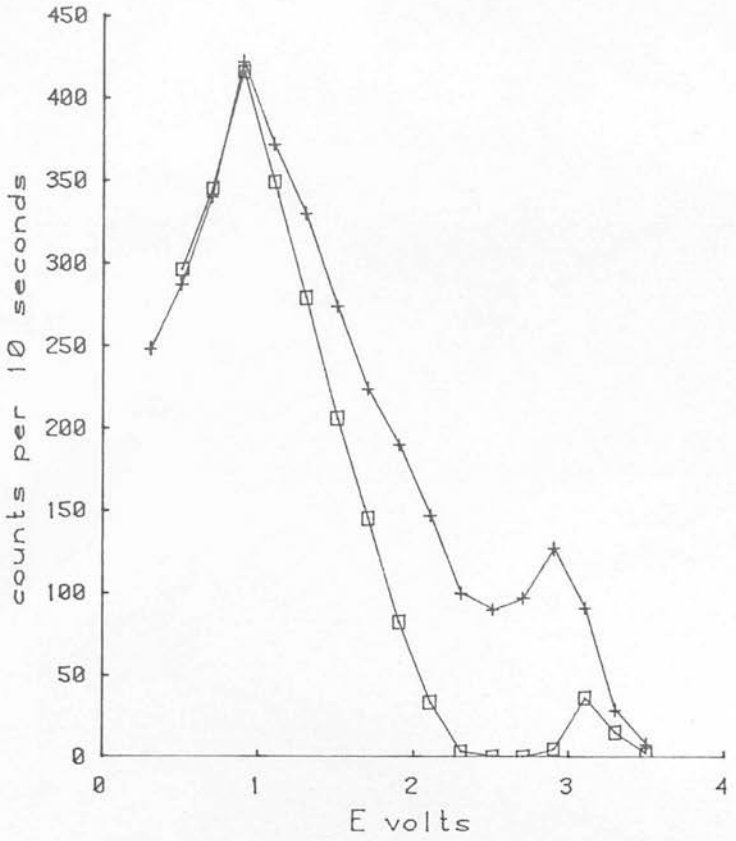

Figure 2.4 Spectrum for P-32

+—+ channel 1 □—□ channel 2

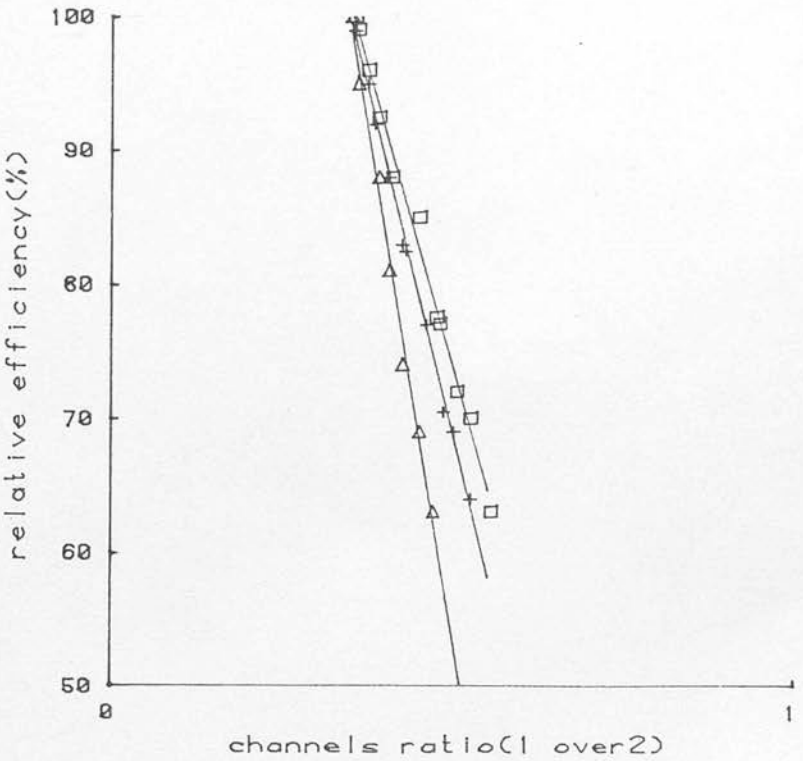

Figure 2.5 Calibration curves for colour quench correction in counting P-32 using sample channels ratio.

△-△ Methyl Orange +-+ Faeces □-□ Urine

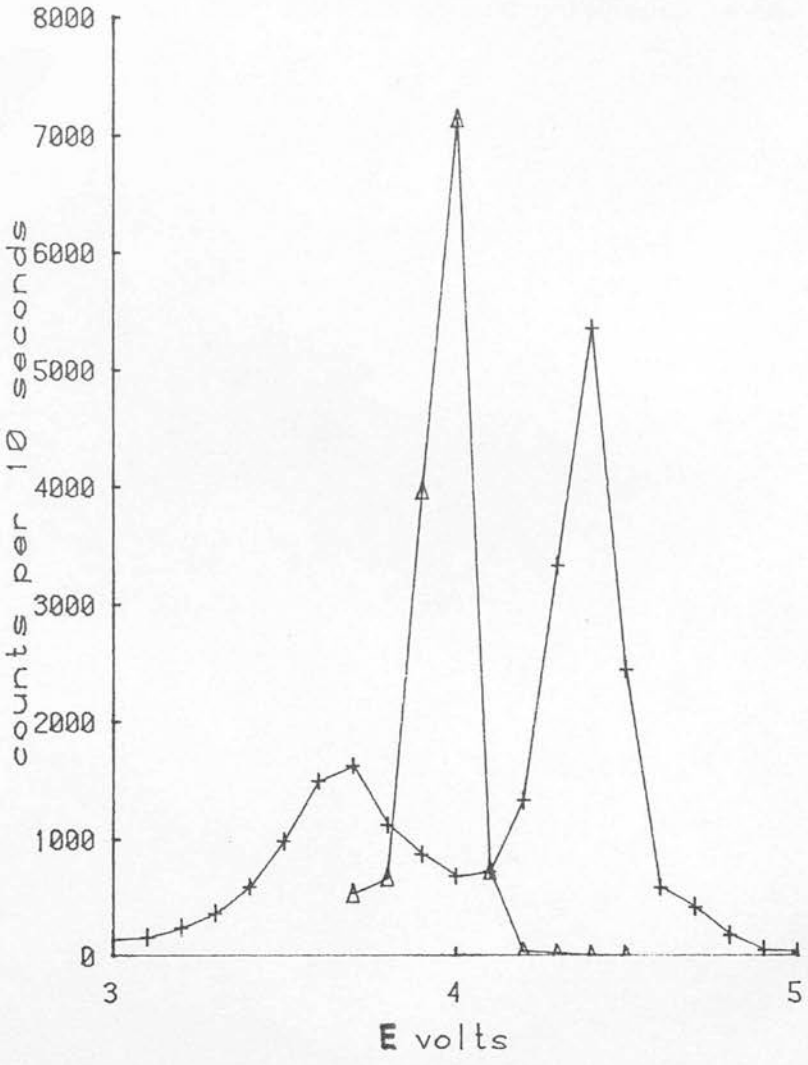

Figure 2.6 Spectra for Cr-51 and Ru-103

△-△ Cr-51 +-+ Ru-103

CHAPTER 3

PRELIMINARY INVESTIGATION INTO THE EFFECT OF INTERNAL PARASITES ON THE METABOLISM OF CALCIUM AND PHOSPHORUS

INTRODUCTION

The prime aims of this study were to ascertain the suitability of the chosen methods for studying changes in metabolism and if possible, the nature and extent of these changes.

ANIMALS

A total of 18 female and nine male lambs, aged four months, were available. After 14 days adjustment to individual pens, all lambs were fitted with ruminal and abomasal cannulae and the female lambs with ileal cannulae. Two female lambs were discarded because of advanced hernias at the site of ileal cannulation.

EXPERIMENTAL DESIGN

The animals were allocated at random within sex to one of three treatment groups: each group contained three males and five or six females and were designated the *Trichostrongylus colubriformis* (TC), *Ostertagia circumcincta* (OC) and control (C) groups. The TC group was given 2,500 infective *T. colubriformis* larvae and the OC group 4,000 infective *O. circumcincta* larvae daily. Dosing started when the animals were five months of age and continued for 14 weeks. Two radioactive balance trials were carried out the first after five weeks (Trial A) and the second four weeks later (Trial B).

At the start of dosing all lambs were offered 1200 g Ruminant A diet per day. Refusals were collected and weighed daily. Lambs showing inappetance for more than three days were killed. Body weights were recorded fortnightly from week five onwards; animals to be included in a balance trial were weighed on a convenient day before and after the trial. The animals were killed 14 weeks after dosing.

BALANCE TRIAL A

Two females with the best appetite were selected from each treatment group for the trial. Appetite was restricted to 800 g food/day, delivered hourly by automatic feeders. Some refusals occurred and were weighed and pooled for each animal over the collection period. The trial period was divided into a five day preliminary period and a 12 day collection period. On the first day of the collection period each sheep was given 3mCi of ^{32}P, dissolved in 5 ml of 9 g sodium chloride/litre, by intravenous injection into the right jugular vein through an in-dwelling nylon catheter (Portex Ltd., Kent, England).

Total daily collection of urine and faeces commenced on the first day of the collection period and continued for the next 12 days. An aliquot of urine and faeces was taken for the determination of SR and a 10% sample, pooled over the collection period, for the determination of dry matter digestibility and the excretion and retention of stable P (^{31}P). Blood samples were taken at 09.00 on days seven, nine, 11 and 13 of the collection period.

Endogenous faecal P excretion was measured by two methods; the first compared the areas under the SR/time curve for faeces and urine (assumed to be the transport form) and the second mean SR of the faeces and urine over the linear part of the SR/time curve.

BALANCE TRIAL B

After nine weeks of dosing seven male animals, three from the OC group and two from the TC and C groups were used. Each animal received 1200 g Ruminant A diet/day by automatic feeder for a total of 12 days, a five day preliminary and a seven day collection period.

On the first day of the collection period a single shot containing 1.3 mCi of ^{32}P and 250 μCi of ^{45}Ca was given intravenously. The endogenous faecal Ca and P was calculated from the ratio of area under the SR/time curve for faeces and plasma. The computer program SAAM (simulation, analysis and modelling) was used to fit a multiexponential equation to the SR/time curve for plasma for both Ca and P (Berman, 1965).

Blood samples were taken frequently by means of heparinised syringes according to the sampling schedule previously described. Faeces and urine were collected daily and 10% taken as a sample.

PEPSINOGEN

Three females and three males from the C group and three females and three males from the OC group were used for a study of plasma pepsinogen levels. From the start of dosing, blood samples were collected every four days for five weeks

and then every eight days until week nine.

CHEMICAL ANALYSIS

Plasma P and Ca concentrations were determined by the automated flow line method. In ashed samples, P was determined by the method based on the Sample Preparation Unit and Ca by titration with potassium permanganate after the precipitation of Ca as calcium oxalate. Plasma pepsinogen levels were determined by the radial diffusion method.

STATISTICAL ANALYSIS

The data were analysed statistically by Student's t test. Means are given in tables and text with 1 SE unit.

RESULTS

HEALTH OF EXPERIMENTAL ANIMALS

The investigation was characterised by a large loss of animals towards the end of the experiment. Of the 25 lambs which started, only 13 completed the observational period. There were two main causes, first the loss of a cannula from the abomasum or ileum and secondly, inappetance in the group which received the T. colubriformis larvae (Table 3.1).

The animals which survived appeared healthy and alert throughout the experiment with no obvious clinical signs of infection. Occasionally the faeces had a "putty" like consistency for two to three days, but the pelleting rapidly returned. All the subsequent qualitative information refers to these animals with the following exception. If an animal had completed a balance trial before death its

results were retained and presented with the balance data.

PARASITOLOGICAL OBSERVATIONS

Egg counts

The mean weekly faecal egg counts are shown in Fig. 3.1 for the OC group. Eggs appeared in the faeces after three weeks of dosing for the OC group, rose to a maximum concentration of 1,362 ± 397 eggs/g fresh faeces after seven weeks and fell to 10 ± 4 by the end of the experiment. The egg counts for each animal at intervals during the experiment are given in Appendix 3.1.

Eggs appeared in the faeces of the TC group after three to four weeks of dosing, when the eggs of an additional species, Haemonchus contortus, were identified. The donor animal for the T. colubriformis infective larvae was replaced but since H. contortus is a more prolific egg layer and no attempt was made to separate the eggs from the two species, no information on the faecal egg counts of this group was presented.

Worm population

The information on worm population comes from two sources, those found when the animals were killed during and at the end of the experiment.

The total number of worms recovered at the end of the experiment are shown in Table 3.2. Of the total larval dose, 20.9 ± 5.6 and 7.8 ± 3.7 per cent were recovered at slaughter in groups TC and OC respectively. The mean worm populations recovered were 47,467 ± 12,701 and 28,450 ± 13,636

for the TC and OC groups respectively. Table 3.2 also shows the number of adult H. contortus found in the TC animals. The recovery and mean worm population was 41 ± 10.9 per cent and 1,333 ± 3.53 respectively.

There was no evidence that the worm burden of the lambs killed during the experiment was greater than those which survived the experiment (Table 3.1).

PATHOLOGY

Table 3.2 gives details of the pathological findings in the 13 animals slaughtered at the end of the experiment. All the OC animals had severe lesions in the abomasum, namely, hypertrophy of the fundic mucosa and presence of lesions specifically associated with the penetration of worm larvae. Severe lesions were also seen in the small intestine of the TC group and were characterised by an atrophic mucosa and parasites partially embedded in the villi. One of the TC animals (No. 441) had no lesions due to H. contortus in the abomasum, although adult worms were found. The remaining two TC animals had lesions, one mild and the other moderately severe.

BODY WEIGHT AND FOOD INTAKE

The change in the mean body weights of the three groups are given in Fig. 3.2. Comparison amongst groups was complicated by the fact that groups differed in numbers of males and females and that the males were heavier than the females. However, between the 5th and 14th week, the C group increased by 3.8 kg, whereas the infected groups increased by less than 1 kg. The body weights of each

animal at intervals during the experiment are given in Appendix 3.2.

A feature of the experiment was the poor appetite exhibited by certain lambs. For some lambs it was because of the direct effect of the operation that they never achieved their preoperation feed intakes even in the control group. The surviving members of the groups were not therefore, completely representative, nor were they allowed to express their full appetite between weeks five to 13, as some of the lambs left no refusals (Appendix 3.3). For these reasons no comparison has been made between treatments, but there was a tendency overall for the infected animals to consume less than the control animals.

CONCENTRATION OF CALCIUM AND PHOSPHORUS IN PLASMA

The mean values for the lambs on balance trials A and B are given in Table 3.3. The plasma P concentrations of the TC group were much lower ($P<0.01$) than either those for the C or OC lambs. No significant differences were found in the P concentrations between the C and OC groups or in the Ca concentrations amongst the groups.

BALANCE TRIALS A AND B

Dry matter digestibility

Although there were large differences in dry matter (DM) intake between the balance trials, the mean DM intakes were 0.635 ± 0.018 and 0.908 ± 0.069 kg/day for balance trials A and B respectively, there was no evidence for any difference amongst treatments within and between balance trials (Table 3.4a, b). The mean DM digestibility in

Trial A was 0.62 ± 0.0095 and in Trial B 0.64 ± 0.0138.

Phosphorus excretion and retention

The data for trials A and B are given in Tables 3.5a and 3.5b respectively.

Interpretation of the data is difficult because of its variability, due in part to variation between animals. Evidence for individual variation is seen in urinary excretion, where values ranged from 0.02 to 0.97 g/day. Furthermore, high urinary excretion was associated with negative retention in Trial A, indicating that part of the parasite effect was direct on bone mineralisation.

Despite this variability, the data suggest that the TC group was most severely affected and the effect of the parasites was less in trial B than in trial A.

BALANCE TRIAL A

Endogenous faecal excretion

Comparison between areas under the SR/time curve for faeces and urine The changes in SR with time after dosing in faeces are given in Fig 3.3 for the three treatments. Maximum SR was seen on day two after dosing and thereafter fell in an exponential manner. Differences between treatments were small, but there was an indication that the area under the SR/time curve was less for the infected groups than the control. Comparing the data for the control animals with that for the three infected animals, which were followed for eleven days, the mean area for the control animals was about 9% greater than that for the infected animals. The changes in SR with time after dosing in faeces for each animal in Balance Trial A are

given in Appendix 3.4.

The corresponding changes in urine to that in faeces are given in Fig. 3.4. As expected maximum SR occurred more rapidly in urine, occurring within the first day. There were large differences in urinary SR between treatments during the first five days after dosing. The SR of urine for the TC group was always much lower than the corresponding values for the C and OC groups. Differences between the C and OC groups were confined to the first day when the value for the OC group was some threefold greater than that for the C group. The changes in SR with time after dosing in urine for each animal in Balance Trial A are given in Appendix 3.5.

A comparison of SR in urine with that in plasma was possible on the 7th and 9th day. It will not be exact as the plasma value refers to one point in time (Table 3.6), whereas the urine value represents the mean for the day's excretion. Despite this limitation good agreement was found for the C and OC groups but not for the TC group; for the latter the SR in urine was from a quarter to a half that for the plasma.

The fraction of faecal P which is of endogenous origin was calculated from the ratio of the area under the SR/time curve for faeces to that in urine. Large differences between treatments were found and reflect the large treatment differences in urinary SR over the initial period after dosing. For the TC group the fact that the area under the curve for urine was less than that for faeces implies that

the SR of urine and of salivary P, the principal source of endogenous faecal P, was not the same in sheep infected with TC worms. For the OC group, the ratio suggested that nearly all the faecal P was of endogenous origin and that nearly all dietary P was absorbed (Table 3.7).

Lofgreen and Kleiber method The fraction of faecal P of endogenous origin was determined by the point method of Lofgreen and Kleiber (1954), using the delay proposed by Lofgreen (1960). In Table 3.5a the endogenous fraction was calculated from the ratio of SR in faeces to that in plasma. The endogenous fraction was also calculated, using a two day delay, from the ratio of SR in faeces to that in urine (Table 3.8). Good agreement was found for the OC group and one of the animals in the C group but not for the TC group and the other animal in the C group. It is interesting to note that the TC animals and animal number 339 in the C group were the ones which excreted very little P in their urine.

The fraction of faecal P of endogenous origin calculated from the ratio of SR in faeces to that in urine, by the point method, differed markedly from the endogenous fraction calculated from the ratio of the area under the SR/time curve for faeces to that in urine (Table 3.7).

BALANCE TRIAL B

Endogenous faecal excretion of phosphorus

SR in plasma The changes in SR with time after dosing followed an exponential decline in all groups with a tendency for the SR in the plasma to be ranked in order of magnitude TC, OC and C (Fig 3.5). The SR/time curve was

found to fit a multiexponential curve of the type

$$SR = x_1 e^{-\alpha_1 t} + x_2 e^{-\alpha_2 t} + x_3 e^{-\alpha_3 t} + x_4 e^{-\alpha_4 t} + C$$

with four terms. The values for x_i at α_i for the individual animals are given in Table 3.9.

The area under the SR/time curve was calculated by three methods 1) triangulation, 2) integration of the multi-exponential curve and 3) by plotting the curve on graph paper and counting the squares. A comparison of areas calculated by the first two methods for the period 0 to four days after dosing is given in Table 3.10 where a small difference in the estimates can be seen. The mean difference (0.184) between the areas calculated by triangulation and integration was significant ($P < 0.025$), but was only about 3% of the overall mean. It probably reflected the problem of estimating the area under the initial part of the curve. The counting method gave very similar values.

SR in faeces The areas under the SR/time curve for faeces are given in Table 3.10 and were very similar for all groups.

Fraction of faecal phosphorus of endogenous origin The values for the fraction were very similar irrespective of what method was used to calculate the area under the plasma curve. The integration method, however, had the advantage that it permits estimates of the areas beyond the exponential period and allows the investigation of the effect of varying delay between secretion of P in saliva and its subsequent excretion in faeces (Table 3.10). It was found that the fraction decreased but was relatively

insensitive to decreasing delay between plasma and faeces. Lofgreen and Kleiber (1954) took the time between dosing and the appearance of maximum SR in faeces as a measure of this delay and with this type of diet a time of two days is usually found (Field et al, personal communication). If we take this as the true delay an error of one day either side would lead to an error of less than 5% in the estimate.

Phosphorus balance trial

Feed refusals were seen in all animals with the exception of number 340 in the control group. Only one lamb, number 440 in the OC group, refused a large proportion (51%) of its diet.

The findings were characterised by large individual differences in excretion, retention and absorption (Table 3.5b) and consequently it was not possible to arrive at any conclusions as to the effect of parasites on P metabolism. It appeared, however, that lambs infected with T. colubriformis absorbed P less effectively, but the quantity absorbed was still greater than the animals' P requirements as judged by the significant faecal excretion of endogenous P.

Endogenous faecal excretion of calcium

SR in plasma The changes in SR with time after dosing showed a similar picture to that of P, but some of the curve could be fitted by a multiexponential equation with only three terms (Table 3.11).

The areas under the curve given in Table 3.12 were calculated by integration of the above multiexponential equations and

it was found that the estimates of the fraction of faecal Ca of endogenous origin were insensitive to delays in excretion. It appeared that the fraction was smaller for the control group than for the infected groups.

SR in faeces The area under the SR/time curve for faeces was much less for the control than the infected groups (Table 3.12). The mean area for the control group was 0.133 mCi/g Ca as compared with areas of 0.239 and 0.259 mCi/g Ca for the TC and OC groups respectively.

Calcium balance trial

Large differences in retention between individuals within the same group were seen (Table 3.13). Negative retentions were, however, confined to the infected group. A significant correlation ($r = 0.944$, $P < 0.01$) was found between retention and the efficiency of absorption of dietary Ca. Endogenous faecal excretion was greater in the TC group, reflecting either poor absorption or enhanced excretion of endogenous Ca.

PEPSINOGEN

The mean plasma pepsinogen concentrations for the C and OC groups are given in Fig 3.6. Mean plasma pepsinogen concentrations increased two weeks after infection in the OC group. The pepsinogen concentrations for the 12 animals at intervals during the experiment are given in Appendix 3.6.

DISCUSSION

HEALTH OF EXPERIMENTAL ANIMALS

One major problem encountered in this preliminary investigation was the high proportion of experimental animals which died

or had to be destroyed during the experiment; out of 25 animals which started only 13 survived to the end of the experiment. There were two main reasons for this. First, there was the loss of cannulae, especially ileal cannulae, which resulted in these animals being destroyed. Secondly, at about week three of the experiment, the TC animals were found to have a concurrent Haemonchus contortus infection.

The main cause of the loss of ileal cannulae was a hernia in the abdominal muscles, probably due to the cannula being placed too low in the flank and to the muscle having insufficient strength at that site in animals of four months of age. Cammell (1977) has recommended that sheep should be selected for surgical preparation at about 12-18 months. One possible cause of the problems with the abomasal cannula was its position. Langlands (private communication) stated that abomasal cannulation in their lambs had produced no difficulties when these cannulae were placed between the ribs in the last intercostal space.

A further problem was the variable nature of the voluntary feed intake. Some animals, following surgical operation, never achieved their preoperational intake, whereas others exhibited temporary loss of appetite lasting two to three days, often associated with the passage of small quantities of very hard pellets. These changes in voluntary intake were attributed to the effects of cannulation as they were seen in both control and infected animals. Small changes in voluntary feed intake consequent upon surgical establishment of gastro-intestinal cannulae have been suggested (MacRae,

1975), but none of such magnitude as found here have been described. MacRae (personal communication) has encountered changes in feed intake of a similar magnitude with cannulated sheep using Ruminant A diet, but not with forage diets.

METHODS FOR DETERMINING ENDOGENOUS EXCRETION AND TRUE ABSORPTION

An important object of the preliminary experiment was to investigate the relative merits of the different methods proposed for the determination of endogenous faecal excretion and true absorption of P. In all two comparisons were investigated: 1) the use of the SR in plasma or urine as a measure of the SR in the transport form and 2) the use of areas or points to calculate the proportion of faecal P of endogenous origin. Unfortunately it was not possible to compare directly the estimates of the point and area methods, the former was used in Balance Trial A and the latter in Balance Trial B.

The use of SR in urine instead of that in plasma presented difficulties and these difficulties were related to the quantity of P excreted in the urine. For the animals which excreted only small quantities of stable and radioactive P in their urine, the errors attached to the values for SR in urine were large and were responsible for the erratic pattern of change in the values for the ratio SR faeces/ SR urine found with the point method (Table 3.8). This was more noticeable in the TC group, where three of the four animals excreted only small quantities of P in their urine (Table 3.5a, b). As the excretion of Ca in urine is negligible, the use of urine to measure the SR of the transport form will be unsatisfactory.

In the point method the value for the fraction of P which is of endogenous origin was very sensitive to the time lag between secretion into and excretion from the digestive tract. The fraction was calculated using a value of one or two days for the time lag, the most common values adopted by past workers (Lofgreen and Kleiber, 1954; Lofgreen, 1960), and the values differed on average by 25% (Table 3.8). These findings emphasise the importance of adopting the correct time lag, yet the only suggestion to its determination is that of Lofgreen (1960) who considered that the time taken to achieve maximum SR in faeces following a single subcutaneous or intravenous dose of ^{32}P was a true measure.

The area method is theoretically the best method. It is insensitive to time lags and uses all the potential data and is therefore more accurate. Three methods were used to calculate the area: 1) Triangulation, 2) integration of the multiexponential equation describing the SR/time curve and 3) counting the graph squares under a plot of the SR/time curve, but all gave comparable values.

The method of triangulation was adopted for the next series of experiments, because of its simplicity and a simple computer program was written for the calculation. Various values for the time delay were introduced into the calculation, but with little effect on the final value. A two day delay was introduced, i.e. the area under the faecal SR/time curve for time $t+2$ was compared with that for the plasma curve for time t, and the value was based on the fact that it took two days for peak faecal SR to be

reached following intravenous administration of ^{32}P.

METABOLIC EFFECTS OF PARASITES

The experimental conditions for producing a parasitic infection of T. colubriformis and O. circumcincta were essentially those used by Coop et al (1976) and Coop et al (1977) and induced similar levels of infection as measured by the number of adult worms recovered at slaughter. Most of the findings reported by Coop et al (1976) and Coop et al (1977) were seen in the present animals, namely a reduction in growth rate but without overt clinical signs of infection, normal plasma concentrations of Ca and P in the OC group and low values for P in the TC group. The elevated plasma pepsinogen concentrations described by Coop et al (1977) were also seen in the present OC group. The poor retention of P shown in Balance Trial A demonstrates that T. colubriformis has a dramatic effect on the mineral metabolism of the host, thus supporting the conclusions of Reveron et al (1974a) and Sykes and Coop (1976b).

The new information, although limited in nature, refers to the endogenous faecal excretion and true absorption of Ca and P in the infected groups. The finding that the absorption of Ca and P by the OC and C groups were similar gives support to the suggestion by Sykes et al (1977) that the poor mineralisation of bone in the OC group is a matrix and not a mineral osteoporosis. On the other hand, with the TC group there was evidence for a reduction in the absorption of P but not Ca. Paradoxically, the endogenous faecal excretion of P was greater than the minimum

endogenous excretion of P by sheep (12-16 mg/kg liveweight per day; ARC, 1980; Field, Dingwall and Munro, personal communication). Since it is now generally recognised that P absorbed in excess of requirements is re-excreted into the digestive tract, this observation suggests that the TC group, even though absorbing P less efficiently, absorbed more P than their requirements. An alternative explanation is that the action of the TC worms in the mucosa increased the rate of loss of P containing mucosal cells and fluids into the lumen of the small intestine. Such losses would not be under the control of the body and would increase the minimum endogenous faecal excretion.

PROPOSED EXPERIMENTATION

A second series of experiments were carried out to confirm the effects of the parasites on the absorption of Ca and P and to attempt to separate the effects of T. colubriformis on absorption and secretion of P in the small intestine.

To circumvent some of the difficulties encountered in the preliminary experiment the animals were older, the abomasal cannulae were placed between the ribs in the last intercostal space and the ileal cannulae higher in the flank. The fraction of Ca and P which is of endogenous origin was calculated by the area method using a two day delay.

In general the animals were kept under management conditions similar to those for the first series, except that more reliable feeders delivering feed at two hourly intervals were utilised.

TABLE 3.1 The total number of worms and the pathological findings in the animals which were killed before the end of the experiment (Series 1)

ANIMAL NO	SEX	WEEK OF DEATH	REASON FOR DEATH	WEIGHT AT DEATH (kg)	EGG COUNT AT DEATH (epg)	TOTAL WORM POPN.	LARVAL INPUT	% RECOVERY	OC LESION IN ABOMASUM	HC LESION IN ABOMASUM	TC LESION IN S INTESTINE
377 TC	F	Taken off experiment during Week 3 as very lame									
410 TC	M	6	Stopped eating	34	1607	43200 TC 1420 HC	99250 TC 3250 HC	43.5 TC 43.7 HC	NIL	SLIGHT	SEVERE
422 OC	F	8	Loss of Ileal Cannula	30	273	2120	210000	1.0	SEVERE	NIL	NIL
387 OC	F	9	Loss of Ruminal Cannula	23.5	4	28800	240000	12.0	SEVERE	NIL	NIL
353 C	M	10	Loss of Abomasal Cannula	37	-	-	-	-	NIL	NIL	NIL
375 TC	F	10	Stopped eating	24.5	3864	30600 TC 1400 HC	159000 TC 3500 HC	19.3 TC 40.0 HC	NIL	SLIGHT	SEVERE
425 TC	F	10	Stopped eating	28	1020	27400 TC 2700 HC	159000 TC 3500 HC	17.2 TC 77.0 HC	NIL	SLIGHT	SEVERE
301 OC	F	10	Loss of Abomasal Cannula	23	15	600	260000	0.2	SEVERE	NIL	NIL
307 TC	F	10	Stopped eating	19.5	16800	101200 TC 3000 HC	161500 TC 3500 HC	62.6 TC 85.7 HC	NIL	SLIGHT	SEVERE
383 C	F	11	Loss of Ileal Cannula	24	-	-	-	-	NIL	NIL	NIL
312 OC	F	11	Loss of Ileal Cannula	26.5	0	17800	284000	6.3	SEVERE	NIL	NIL
291 C	F	12	Loss of Ileal Cannula	27.0	-	-	-	-	NIL	NIL	NIL

OC:- Ostertagia circumcincta HC:- Haemonchus contortus TC:- Trichostrongylus colubriformis

TABLE 3.2 The total number of worms and the pathological findings at slaughter for the 13 animals killed at the end of the experiment (Series 1)

ANIMAL NO	SEX	WEEK OF DEATH	TOTAL WORM POPN.	LARVAL INPUT	% RECOVERY	OC LESION IN ABOMASUM	HC LESION IN ABOMASUM	TC LESION IN S INTESTINE
362 C	M	14	-	-	-	NIL	NIL	NIL
394 C	F	14	-	-	-	NIL	NIL	NIL
339 C	F	14	-	-	-	NIL	NIL	NIL
431 C	F	14	-	-	-	NIL	NIL	NIL
309 C	F	14	-	-	-	NIL	NIL	NIL
340 C	M	14	-	-	-	NIL	NIL	NIL
441 TC	M	14	36000 TC 1200 HC	227500 TC 3250 HC	15.8 TC 36.9 HC	NIL	NIL	SEVERE
423 TC	M	14	72800 TC 800 HC	227500 TC 3250 HC	32.0 TC 24.6 HC	NIL	SLIGHT	SEVERE
350 TC	F	14	33600 TC 2000 HC	227500 TC 3250 HC	14.8 TC 61.5 HC	NIL	MODERATELY SEVERE	SEVERE
440 OC	M	14	43200	364000	11.9	SEVERE	NIL	NIL
295 OC	F	14	59400	364000	16.3	SEVERE	NIL	NIL
427 OC	M	14	3200	364000	0.9	SEVERE	NIL	NIL
352 OC	M	14	8000	364000	2.2	SEVERE	NIL	NIL

TABLE 3.3 Mean concentrations of plasma calcium and phosphorus (mg/l) for groups C, TC and OC in Balance Trial A and B (Series 1)

	Ca		P	
	A	B	A	B
C	97.0 ± 3.5	99.9 ± 2.0	73.4 ± 12.9	68.9 ± 4.2
TC	98.6 ± 3.4	95.2 ± 0.95	27.9 ± 1.9	34.2 ± 9.5
OC	86.8 ± 0.20	97.9 ± 1.5	86.0 ± 10.9	55.1 ± 4.8

TABLE 3.4a Digestibility of D.M. for each animal in Balance Trial A (Series 1)

SHEEP NO	LIVE-WEIGHT (kg)	FEED INTAKE (g/d)	DM INTAKE (g/d)	FAECAL OUTPUT FRESH WT (g/d)	FAECAL OUTPUT DRY WT (g/d)	DIGESTIBILITY OF DM
339 C	27.0	793	670	915	267	0.60
394 C	27.0	695	586	600	206	0.65
425 TC	31.0	691	583	797	213	0.63
307 TC	19.75	799	674	1107	275	0.59
301 CC	24.0	741	625	892	250	0.60
422 CC	30.0	798	674	894	250	0.63

TABLE 3.4b Digestibility of D.M. for each animal in Balance Trial B (Series 1)

SHEEP NO	LIVE-WEIGHT (kg)	FEED INTAKE (g/d)	DM INTAKE (g/d)	FAECAL OUTPUT FRESH WT (g/d)	FAECAL OUTPUT DRY WT (g/d)	DIGESTIBILITY OF DM
340 C	38.0	1167	993	886	340	0.66
362 C	36.0	1169	994	1099	374	0.62
441 TC	33.0	1194	1016	872	344	0.66
423 TC	34.0	1101	937	1557	399	0.57
427 OC	40.25	1200	1021	1065	338	0.67
352 OC	34.5	1037	882	856	324	0.63
440 OC	33.5	602	512	336	169	0.67

TABLE 3.5a

Excretion & Retention of Phosphorus by the animals in Balance Trial A (Series 1)

SHEEP NO	FEED INTAKE (g/d)	PLASMA CONCN. (mg/100ml)	PHOSPHORUS INTAKE (g/d)	PHOSPHORUS IN FAECES (g/d)	PHOSPHORUS IN URINE (g/d)	PHOSPHORUS RETENTION	SR_F/SR_P *	ENDOGENOUS FAECAL EXCRETION (g/d)	ENDOGENOUS FAECAL EXCRETION (mg/kg)	TRUE ABSORPTION
339 C	793	6.05	3.84	3.75	0.020	0.070	0.50	1.88	70	0.51
394 C	695	8.62	3.37	2.44	0.45	0.48	0.63	1.54	57	0.73
425 TC	691	2.97	3.35	3.75	0.12	-0.52	0.35	1.31	42	0.27
307 TC	799	2.60	3.87	4.28	0.044	-0.45	0.29	1.24	63	0.21
301 OC	741	7.51	3.59	3.37	0.66	-0.44	0.43	1.45	60	0.47
422 OC	798	9.69	3.87	2.65	0.97	0.25	0.33	0.87	29	0.54

* Calculated using the point method of Lofgreen & Kleiber (1954) using a two day delay

TABLE 3.5b Excretion & Retention of Phosphorus by the animals in Balance Trial B (Series 1)

SHEEP NO	FEED INTAKE (g/d)	PLASMA CONCN. (mg/100ml)	PHOSPHORUS				SR_F/SR_P *	ENDOGENOUS FAECAL EXCRETION		TRUE ABSORPTION
			INTAKE (g/d)	IN FAECES (g/d)	IN URINE (g/d)	RETENTION		(g/d)	(mg/kg)	
340 C	1200	7.30	6.05	5.22	0.13	0.70	0.46	2.40	63	0.53
362 C	1169	6.47	5.90	5.28	0.27	0.35	0.37	1.95	54	0.44
441 TC	1194	4.36	6.02	5.36	0.022	0.64	0.32	1.72	52	0.40
423 TC	1153	2.47	5.82	5.97	0.043	-0.19	0.25	1.49	44	0.23
427 OC	1026	4.59	5.18	4.68	0.062	0.44	0.31	1.45	36	0.38
352 OC	1083	6.20	5.47	3.62	0.92	0.93	0.39	1.41	41	0.60
440 OC	585	5.75	2.95	2.26	0.83	-0.14	0.40	0.90	27	0.54

* Areas calculated by triangulation
Fraction calculated using a two day delay

TABLE 3.6 Phosphorus: Plasma SR (μCi/G) on day 7,9,11,13 after dosing for the individual animals in Balance Trial A (Series 1)

	339 C	394 C	425 TC	307 TC	301 CC	422 CC
DAY 7	17.6	16.2	25.3	27.9	13.4	15.1
DAY 9	16.0	14.6	17.8	21.1	11.3	12.4
DAY 11	14.3	12.3	11.2	17.9	7.7	8.2
DAY 13	8.0	6.4	6.6	12.6	7.8	-

TABLE 3.7 Fraction of faecal P which is of endogenous origin in Balance Trial A. (Series 1)

TREATMENT GROUP	SHEEP No.	COLLECTION PERIOD (Days)	AREA		SR_F/SR_u
			FAECES	URINE	
C	339	11	3.060	3.230	0.95
	394	11	2.950	3.070	0.96
TC	425	11	2.820	0.911	-
	307	11	2.690	1.360	-
OC	301	11	2.760	4.220	0.65
	422	10	2.580	4.050	0.64

TABLE 3.8 Fraction of P which is of endogenous origin calculated using the point method of Lofgreen & Kleiber (1954) using either a one or two day delay for Balance Trial A (Series 1)

		339 C	394 C	425 TC	307 TC	301 OC	422 OC
URINE*	DELAY (D)						
	1	0.46	0.73	-	0.60	0.55	0.45
	2	0.40	0.61	-	0.50	0.42	0.32
PLASMA**	1	0.58	0.73	0.49	0.35	0.55	0.46
	2	0.50	0.63	0.35	0.29	0.43	0.33

* $\frac{SR_F\ (t+D)}{SR_u\ (t)}$ ** $\frac{SR_F\ (t+D)}{SR_P\ (t)}$

TABLE 3.9 Terms x_i and α_i in the multiexponential curve relating plasma SR and time after dosing for phosphorus in Balance Trial B (Series 1)

TREATMENT	SHEEP NO	x_1	α_1	x_2	α_2	x_3	α_3	x_4	α_4
C	340	3.9943	-18.5106	1.2289	-1.0274	0.1630	-0.1473	0.04952	-0.01603
C	362	1.6475	- 8.0476	1.6953	-1.4354	0.1868	-0.2473	0.06054	-0.01921
OC	352	1.4795	- 3.3464	1.0506	-0.8246	0.1552	-0.2325	0.04982	-0.01652
OC	440	3.1802	-11.8268	1.7026	-1.3087	0.2456	-0.2201	0.06584	-0.01341
OC	427	7.5275	-23.4871	2.2043	-1.4904	0.2080	-0.2174	0.05115	-0.01518
TC	441	3.1876	- 8.1643	2.0301	-1.2654	0.2090	-0.2060	0.06403	-0.01804
TC	423	10.1663	- 9.7449	2.9971	-1.9365	0.3393	-0.2590	0.06606	-0.01266

TABLE 3.10

Phosphorus: Areas under the SR/time curve for faeces and plasma calculated by triangulation (T) and integration (I) of the multiexponential curve for the individual animals in Balance Trial B (Series 1)

TREATMENT	SHEEP NO	FAECES AREA (F)	PLASMA AREA					RATIO OF AREAS			
		7 days	4 days I(1)	4 days T	5 days I(2)	6 days I(3)	7 days I(4)	$F/_{(1)}$	$F/_{(2)}$	$F/_{(3)}$	$F/_{(4)}$
C	340	2.56	4.94	4.91	5.16	5.30	5.40	0.52	0.50	0.48	0.47
C	362	2.11	4.76	4.71	4.98	5.09	5.17	0.44	0.42	0.41	0.41
OC	352	2.02	4.78	4.69	4.99	5.12	5.21	0.42	0.40	0.39	0.39
OC	440	2.96	6.24	6.06	6.62	6.89	7.08	0.47	0.45	0.43	0.42
OC	427	2.35	5.34	5.05	5.58	5.74	5.86	0.44	0.42	0.41	0.40
TC	441	2.19	5.93	5.77	6.15	6.30	6.39	0.37	0.36	0.35	0.34
TC	423	2.25*	7.57	7.10	7.98	8.28	8.50	0.29	0.27	0.26	0.26

* Based on daily values

TABLE 3.11 Terms x_i and α_i in the multiexponential curve relating plasma SR and time after dosing for calcium in Balance Trial B (Series 1)

TREATMENT	SHEEP NO	x_1	α_1	x_2	α_2	x_3	α_3	x_4	α_4
C	340			0.1947	-1.8495	0.1342	-0.1829	0.03466	-0.02561
C	362*			0.2905	-3.0833	0.1422	-0.1846	0.02024	-0.01308
OC	352			0.2058	-0.9489	0.1008	-0.1666	0.04167	-0.02506
OC	440	0.3254	-3.3914	0.1591	-0.3442	0.03727	-0.09081	0.04027	-0.02076
OC	427			0.1288	-0.8637	0.1054	-0.1765	0.03136	-0.02095
TC	441*			0.1972	-1.5011	0.1438	-0.2536	0.05108	-0.04388
TC	423			0.1581	-0.3058	0.04040	-0.06624	0.01244	-0.01485

* Data only up to 30 hours

TABLE 3.12

Calcium: Areas under the SR/time curve for faeces and plasma calculated by integration of the multiexponential curve for the individual animals in Balance Trial B (Series 1)

TREATMENT	SHEEP NO	FAECES AREA (F)	PLASMA AREA				RATIO OF AREAS			
		7 days	4 days	5 days	6 days	7 days	$F/_{P(4)}$	$F/_{P(5)}$	$F/_{P(6)}$	$F/_{P(7)}$
C	340	0.136	2.07	2.13	2.16	2.17	0.066	0.064	0.063	0.063
C	362	0.130	1.97	2.09	2.18	2.24	0.066	0.062	0.060	0.058
OC	352	0.222	2.34	2.40	2.44	2.46	0.095	0.092	0.091	0.090
OC	440	0.345	2.64	2.75	2.81	2.85	0.130	0.126	0.122	0.121
OC	427	0.210	2.05	2.12	2.17	2.20	0.103	0.099	0.097	0.095
TC	441	0.212	1.84	1.85	1.86	1.86	0.115	0.114	0.114	0.114
TC	423	0.266	1.85	1.91	1.95	1.98	0.144	0.139	0.136	0.134

TABLE 3.13 Excretion & Retention of calcium by the animals in Balance Trial B (Series 1)

SHEEP NO.	FEED INTAKE (g/d)	PLASMA CONCN. (mg/100ml)	CALCIUM INTAKE (g/d)	CALCIUM IN FAECES (g/d)	CALCIUM RETENTION	SR_F/SR_P*	ENDOGENOUS FAECAL EXCRETION (g/d)	ENDOGENOUS FAECAL EXCRETION (mg/kg)	TRUE ABSORPTION
340 C	1200	10.19	9.27	8.13	1.14	0.064	0.52	13.5	0.18
362 C	1169	9.79	9.03	8.42	0.61	0.062	0.52	14.4	0.12
441 TC	1194	9.42	9.23	7.98	1.25	0.114	0.91	27	0.23
423 TC	1153	9.61	8.91	9.17	-0.26	0.139	1.27	37	0.11
427 OC	1026	9.82	7.93	6.50	1.43	0.099	0.64	16	0.26
352 OC	1083	10.03	8.37	7.94	0.43	0.092	0.73	21	0.14
440 OC	585	9.51	4.52	5.29	-0.77	0.126	0.67	20	-0.02

* Area calculated by integration of the multiexponential curve
Fraction calculated using a two day delay

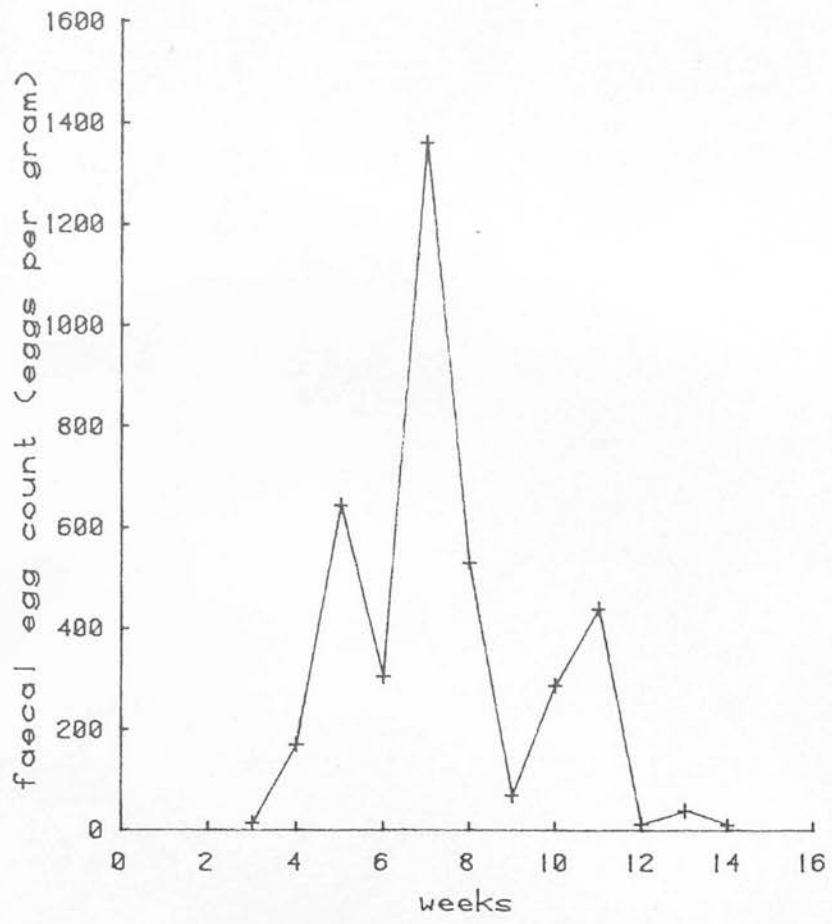

Figure 3.1 The mean weekly faecal egg counts for the OC group (series 1).

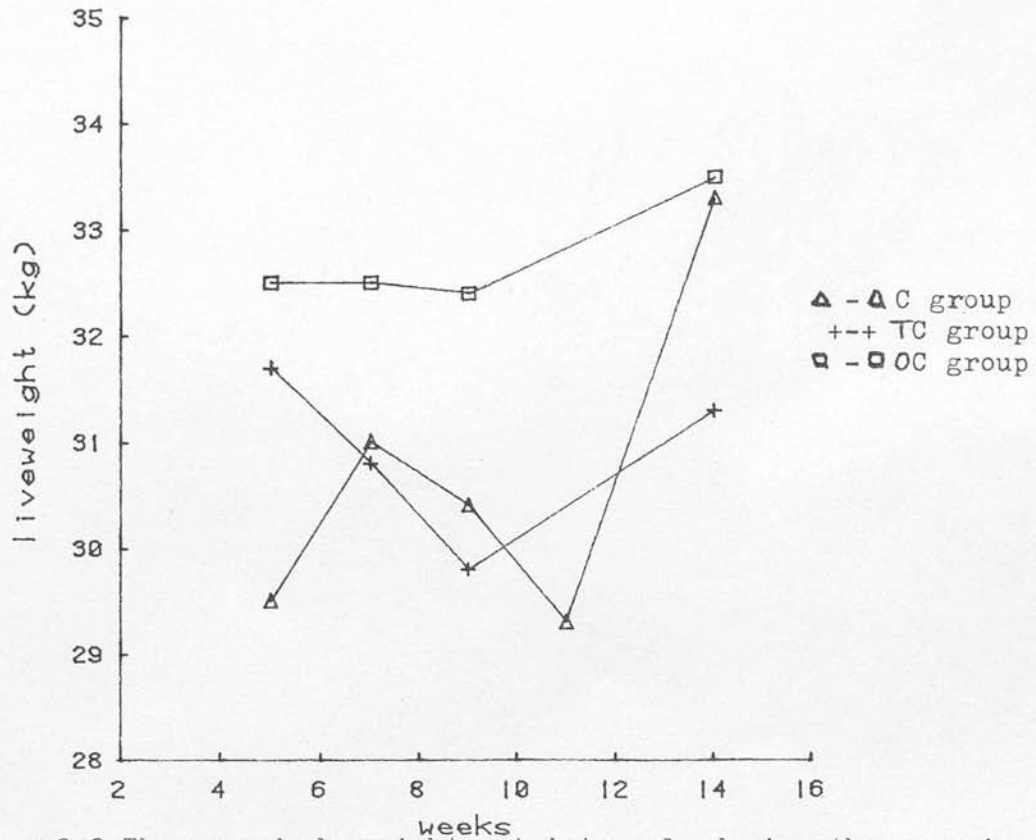

Figure 3.2 The mean body weights at intervals during the experiment (series 1) for groups C, TC and OC.

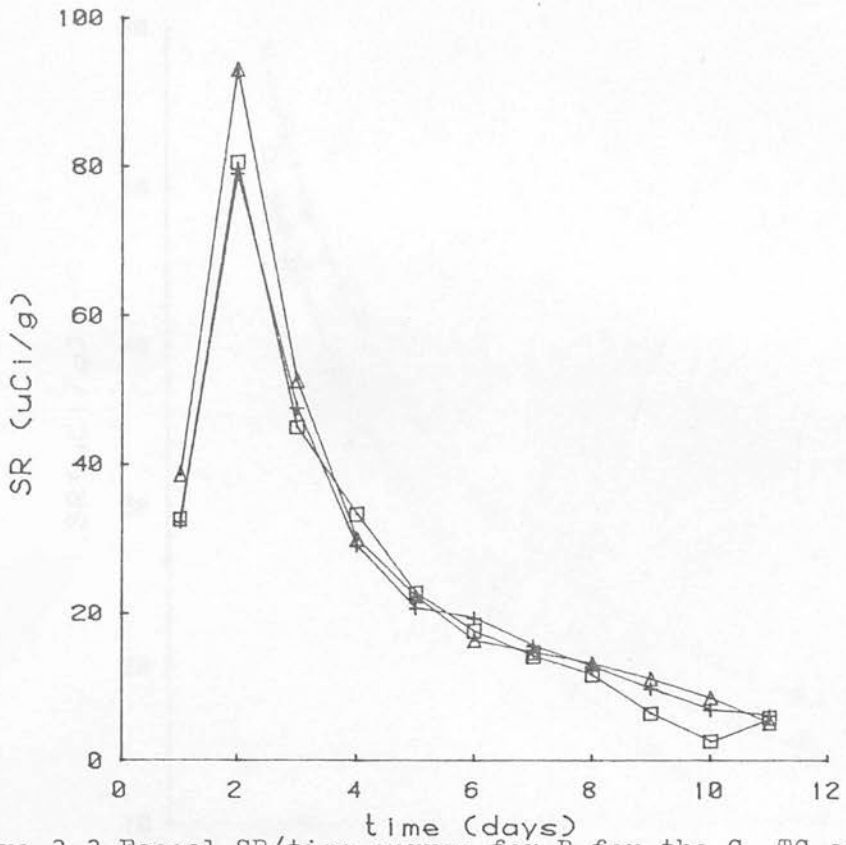

Figure 3.3 Faecal SR/time curves for P for the C, TC and OC groups in Balance Trial A (series 1).
△-△ C group +-+ TC group □-□ OC group

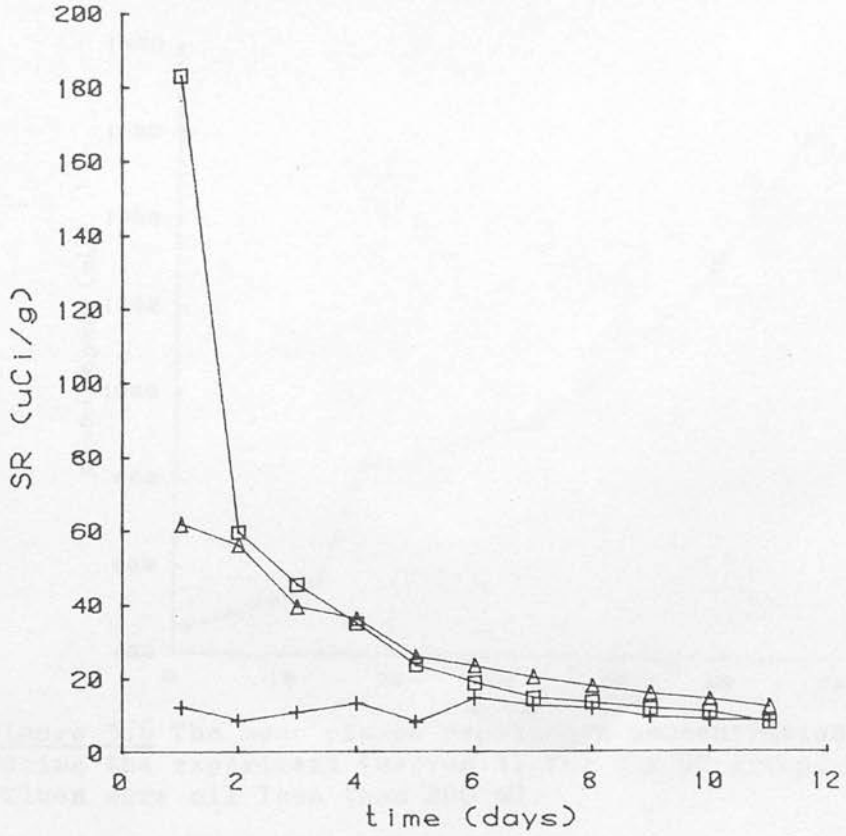

Figure 3.4 Urinary SR/time curves for P for the C, TC and OC groups in Balance Trial A (series 1).
△-△ C group +-+ TC group □-□ OC group

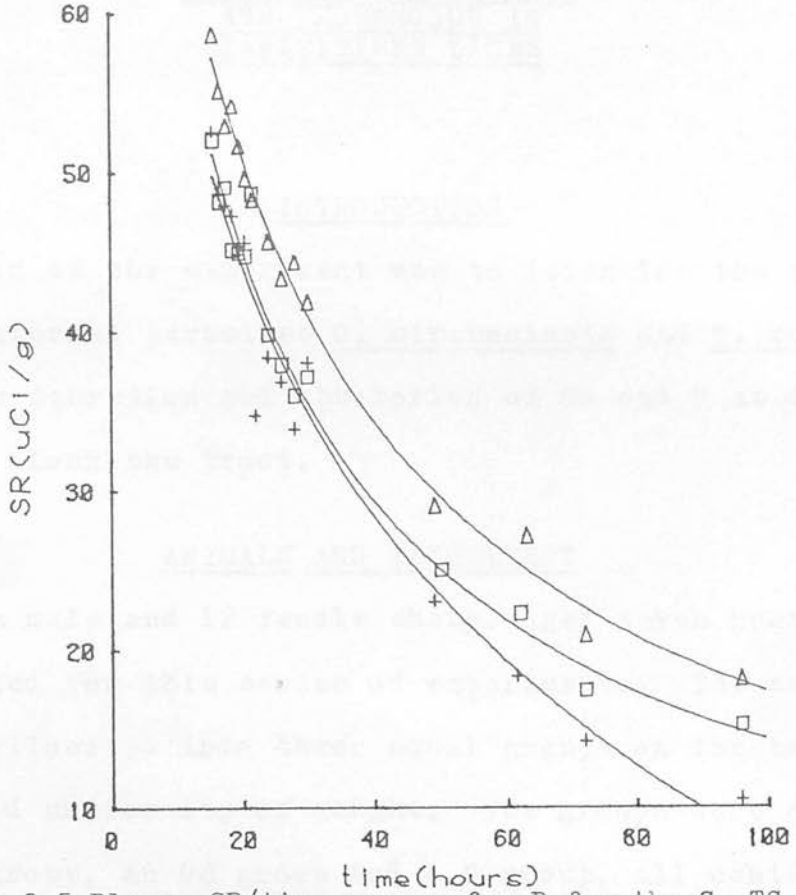

Figure 3.5 Plasma SR/time curves for P for the C, TC and OC groups in Balance Trial B (series 1).
+-+ C group △-△ TC group □-□ OC group

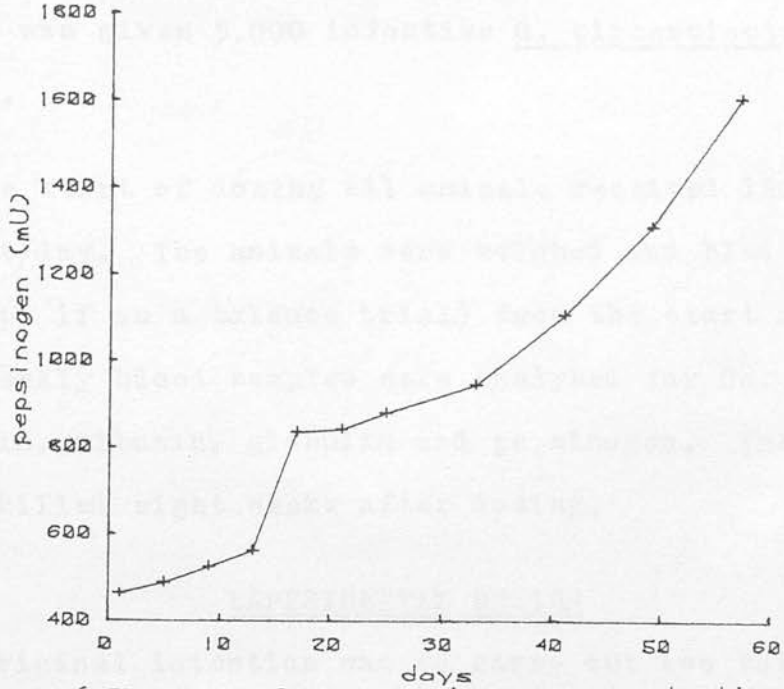

Figure 3.6 The mean plasma pepsinogen concentrations at intervals during the experiment (series 1) for the OC group. The control values were all less than 200 mU.

CHAPTER 4

METABOLISM OF CALCIUM AND PHOSPHORUS IN PARASITISED LAMBS

INTRODUCTION

The aim of the experiment was to determine the effect of two internal parasites O. circumcincta and T. colubriformis on the secretion and absorption of Ca and P at different sites along the tract.

ANIMALS AND MANAGEMENT

Twelve male and 12 female sheep, aged seven months, were selected for this series of experiments. The animals were allocated into three equal groups on the basis of sex and uniformity of weight. The groups were as before: a TC group, an OC group and a C group, all containing four males and four females. The TC group was given, daily, 3,000 infective T. colubriformis larvae and the OC group was given 5,000 infective O. circumcincta larvae daily.

At the start of dosing all animals received 1200 g Ruminant A diet/day. The animals were weighed and bled weekly (except if on a balance trial) from the start of dosing. The weekly blood samples were analysed for Ca, P, total protein, albumin, globulin and pepsinogen. The animals were killed eight weeks after dosing.

EXPERIMENTAL DESIGN

The original intention was to carry out two balance trials, the first with animals infected with O. circumcincta and

the second with animals infected with T. colubriformis; the timing of the balance trials to be chosen to coincide with the maximum damage by the parasite, three weeks for the stomach and six weeks for the intestinal parasite.

The poor appetite of some of the lambs forced changes in this scheme, the most important of which was the necessity for an extra balance trial to obtain control data at the dietary intake achieved by the poor eaters in the infected group.

Details of the three balance trials are given in Table 4.1.

Problems were encountered in trial B with food intake. Three of the TC group (759, 838, 828) ate 1000 g food/day and the OC animal (852) 700 g/day. The remaining three animals in the TC group were fed, through the rumen, small amounts of diet to make their total intake 700 g food/day. All animals were given the diets at two hourly intervals by automatic feeders.

On the first day of the collection period a single shot containing ^{32}P and ^{45}Ca was given intravenously into the right jugular vein. In trials A and C the intraruminal infusion of markers was started on the first day of the collection period and in Trial B on the first day of the preliminary period. Samples of digesta from the rumen, abomasum and ileum were taken on the last two days of the infusion period to form a composite sample. The procedure adopted in Trial B avoided the handling of the cannulae during the collection period and was an unsuccessful attempt

to reduce the incidence of variable appetite during the collection period.

Blood samples were taken by means of heparinised vacutainers as previously described. Samples of digesta from the abomasum and ileum were taken at 3,6, 9 and 12 hours after dosing and then once daily. A 10 or 20% sample of faeces and urine was collected daily. pH determinations were carried out on the individual unspun abomasal digesta samples.

The endogenous faecal Ca and P was calculated from the ratio of areas under the SR/time curve for faeces and plasma. The fraction of Ca and P of endogenous origin in digesta was calculated using the areas under the SR/time curve for digesta and plasma. Examples of the SR/time curves are given in Fig 4.1a and 4.1b for P and Ca respectively.

CHEMICAL ANALYSIS

The weekly plasma P, total protein and albumin concentrations were determined by automated flow line methods. All other plasma P concentrations and the P concentrations of ashed samples were determined by the Sample Preparation Unit method. All Ca concentrations were determined by atomic absorption. Plasma pepsinogen levels were determined by the radial diffusion method.

STATISTICAL ANALYSIS

Statistical analysis of the data was by an analysis of variance. A regression model for a two-factor analysis of variance was used (Neter and Wasserman, 1974) because the groups were of unequal size. Means are given in tables

with 1 SD unit and in text with 1 SE unit.

RESULTS

CLINICAL OBSERVATIONS

In this series of experiments only one control animal was killed before the end of the experimental period. This was due to inappetance.

The animals appeared healthy throughout the experiment, with no obvious outward signs of infection. Diarrhoea did not occur in any of the sheep.

PARASITOLOGICAL OBSERVATIONS

Egg counts

The mean weekly faecal egg counts are shown in Fig 4.2a and 4.2b for groups TC and OC respectively. Eggs appeared in the faeces after three weeks of dosing for the TC group, rose to a maximum concentration of 1529 ± 193 eggs/g fresh faeces after five weeks and fell to 762 ± 134 after eight weeks of dosing. The high egg count for animal no 813 during the last two weeks of the experiment was due to a marked reduction in food intake.

Worm eggs appeared in the faeces after three weeks of dosing for the OC group and reached a maximum concentration of 527 ± 235 eggs/g fresh faeces after seven weeks.

The egg counts for each animal at intervals during the experiment are given in Appendix 4.1.

Worm population

The total number of worms recovered at slaughter are shown in Table 4.2. Of the total larval dose, 15.5 ± 3.5 and 12.4 ± 2.4 per cent were recovered at slaughter in groups

TC and OC respectively. The mean worm populations at slaughter were 22375 ± 5068 and 29737 ± 5858 for the TC and OC groups respectively.

PATHOLOGY

Table 4.2 gives details of pathological findings at slaughter. All the OC animals had severe ostertagiasis. In general, the animals in the TC group had very severe trichostrongylosis. Animal no 775 showed subtotal villous atrophy and there was evidence of immunity suggested by the presence of large numbers of subepithelial and intra-epithelial globule leucocytes. The jejunum of animal no 765 showed subtotal villous atrophy but the worms were very numerous and the duodenum was severely damaged and flattened.

BODY WEIGHT AND FOOD INTAKE

The change in the mean body weights of the three groups are given in Fig 4.3. Each group lost weight during the experiment and the overall weight loss was 1.7, 2.2 and 3.1 kg for the C, TC and OC groups respectively. The body weights of each animal at intervals during the experiment are given in Appendix 4.2.

A feature of the experiment was the poor appetite exhibited by certain lambs. For this reason no comparison has been made between treatments. The mean daily food intakes for each animal are given in Appendix 4.3.

PLASMA CONSTITUENTS

Calcium and phosphorus

The mean concentrations of plasma Ca and P for each group are shown in Fig 4.4 and 4.5 respectively. The plasma P concentrations of the TC group were much lower ($P < 0.01$) than either those for the C or OC group. The plasma P concentrations fell from 68.3 ± 2.9 to 36.7 ± 2.9 mg/l at five weeks of dosing for the TC group. No significant differences were found in the P concentrations between the C and OC groups or in the Ca concentrations amongst the groups. The concentrations of plasma Ca and P for each animal at intervals during the experiment are given in Appendix 4.4.

Total protein, albumin and globulin

The mean concentrations of plasma total protein, albumin and globulin for each group are shown in Fig 4.6, 4.7 and 4.8 respectively.

The plasma total protein concentrations of the OC group were lower ($P < 0.05$) than either those for the C or TC groups. The plasma total protein concentrations fell from 75.4 ± 1.7 to 63.2 ± 1.9 g/l over the period from week three to week seven of dosing for the OC group.

The plasma albumin concentrations of the TC group were lower ($P < 0.05$) than either those for the C or OC groups. No significant differences were found in the albumin and globulin concentrations between the C and OC groups or in the total protein and globulin concentrations between the C and TC groups.

Pepsinogen

The mean plasma pepsinogen concentrations for each group

are shown in Fig 4.9. Mean plasma pepsinogen concentrations for the OC group increased two weeks after infection and reached a maximum of 563± 109 mU at seven weeks of dosing. No significant differences were found in the pepsinogen concentrations between the C and TC groups. The plasma pepsinogen concentrations of the OC group were higher ($P < 0.05$) than either those for the C or TC groups.

FLOW OF DIGESTA AND ITS COMPONENTS

Water, dry matter and organic matter

A feature of the results was the high flow of digesta in the abomasum in the OC group (Table 4.3). Although the greater part of the increased flow was water an increase ($P < 0.01$) in flow of DM, but not OM, was observed. The site of the increased DM flow was the liquid phase and the increase comprised 12 and 61 g/d of OM and ash respectively. Sodium (Na) salts formed a large part of the additional ash, since an extra 24 g Na/day was observed to flow in the liquid phase of the OC group (Table 4.3). The flows of water and ash in the OC group were within the absorptive capacity of the small intestine, and by the terminal ileum the flow for the OC and C groups were similar.

Although the flow of abomasal and ileal digesta was similar in the TC and C groups, there was a significant decrease ($P < 0.01$) in the flow of OM in the solid phase of the digesta in the TC group. This reduced flow was responsible for the significant but small increase ($P < 0.05$) in the digestibility of DM and OM at the abomasum, ileum and rectum.

The DM digestibilities were 0.64, 0.67 and 0.65 for the C, TC and OC groups respectively (Table 4.4a, 4.4b). The corresponding figures for OM digestibilities were 0.65, 0.70 and 0.67 (Table 4.5a, 4.5b).

The flows of water, DM and OM were proportional to the level of feeding and consequently no differences attributable to the level of feeding were seen in the digestibility of any component of the digesta at the abomasum, ileum or faeces.

The flow rates of digesta and the flow rate of DM and OM along the digestive tract of the lambs are given in Appendix 4.5.

Phosphorus

The pooled data on flows and efficiency of absorption of endogenous and exogenous P along the digestive tract from the three trials are given in Table 4.6.

There was no evidence that Ostertagia infection had any effect on P secretion into or absorption from the digestive tract and for statistical purposes the data for the OC and C groups were sometimes combined.

Compared with the C group the flow of endogenous P in the TC group was significantly less ($P < 0.001$) at the abomasum, but similar at the ileum at each level of feeding. The net result was that the apparent absorption of endogenous P in the small intestine was only about half that of the C group. The endogenous faecal excretion, whether expressed in absolute quantities or in proportion to liveweight, was

similar for both groups of animals. Endogenous P was absorbed from the region between the abomasum and rectum with an efficiency of 0.35 and 0.80 for the TC and C groups respectively and the difference was significant at the 1% level.

Absorption of dietary or exogenous P occurred mainly in the small intestine; the flow of P in the diet and exogenous P at the abomasum were not significantly different within groups, as were the corresponding flows at the ileum and rectum. The exogenous faecal excretion of the TC group was higher and hence the efficiency of absorption lower ($P < 0.01$) than in the C group; the mean overall values for the efficiency of absorption were 0.34 and 0.60 respectively.

Absorption of endogenous P, unlike exogenous P, appeared to occur both in the small intestine and in the region between the caecum and rectum, the efficiency of absorption of endogenous P was significantly greater ($P < 0.001$) overall than in the small intestine alone.

The flows of endogenous and exogenous P along the gut were proportional to the intake of dietary P and hence there was no effect of level of feeding on the efficiency of absorption of either source of P within a group.

The urinary excretion and plasma concentration of P was significantly less ($P < 0.001$) for the TC than the C animals (Table 4.6).

The flows of salivary P in the C, TC and OC groups are

shown in Table 4.7. The flow of salivary P was reduced in the TC animals.

The flows of P along the digestive tract of the lambs are given in Appendix 4.6.

Calcium

The pooled data on the flows and efficiency of absorption of Ca along the digestive tract are given in Table 4.8.

The effect of the treatments was confined to the endogenous faecal excretion and was more significant when expressed on a liveweight basis. The endogenous faecal excretions of the TC and OC groups were similar, independent of the level of feeding and equal to 18.8 and 17.5 mg/kg liveweight respectively. The values for the C group were greater ($p < 0.01$) at the higher than lower level of feeding, (13.0 and 8.2 mg/kg liveweight respectively) and consequently the parasitic effect was greater ($p < 0.001$) at the lower level of feeding. The endogenous flow of Ca at the abomasum was very small and at the ileum was not significantly different from that in faeces.

The flows of Ca along the digestive tract of the lambs are given in Appendix 4.7.

DISCUSSION

This work has confirmed that *T. colubriformis* and *O. circumcincta* can reduce animal performance without producing any outward clinical signs of parasitism. The experimental conditions for producing a parasitic infection of *T. colubriformis* and *O. circumcincta* were essentially

those used by Coop *et al* (1976; 1977), although the experimentation time in the present study was shorter. The total number of worms recovered at slaughter for both the TC and OC animals were in good agreement with the findings of other workers (Coop *et al*, 1976; 1977). Therefore it is reasonable to suppose that poor growth and mineralisation of the skeleton was taking place.

Sykes *et al* (1977) have argued on the basis of bone pathology and normal plasma concentrations of Ca and P that the osteoporosis associated with chronic *O. circumcincta* is probably a matrix osteoporosis resulting from an energy or protein deficiency. The results of the work in this thesis support this hypothesis in that no apparent effect of *Ostertagia* parasitism on the absorption of Ca and P was seen.

Sykes *et al* (1973) have shown that a protein deficiency has a greater effect than a Ca deficiency on bone mineralisation in pregnant ewes. Sykes and Coop (1977) found that there was a reduction in N digestibility due to abomasal parasitism. This reduction in N digestibility could result from 1) reduced digestion of exogenous or endogenous protein or from 2) increased endogenous losses into the alimentary tract, such as leakage of plasma proteins, digestive secretions or cell sloughing.

A reduction in protein digestion could result from the loss of differentiation and function of acid - secreting parietal cells and pepsinogen - secreting chief cells which occurs after infection of sheep with *O. circumcincta*

(Armour et al, 1966; McLeay et al, 1973; Coop et al, 1977). Plasma pepsinogen concentrations of the OC group were elevated thus supporting the findings of Coop et al (1977). The conversion of pepsinogen to the active proteolytic enzyme pepsin is extremely slow at pH greater than 3.5-4.0 (Hirschowitz, 1957). The pH of abomasal contents from two sheep in the OC group was greater than 3.5 (Table 4.9). The data of Jennings et al (1966) and Anderson et al (1966) suggest that the increases in plasma pepsinogen concentrations seen in the OC animals reflect abomasal damage and an increase in abomasal pH sufficient to curtail pepsin production.

A reduction in protein digestion is an unlikely explanation for a protein deficiency as the correlation between the weight gain and N digestibility differences between the infected and control sheep of Sykes and Coop (1977) was extremely poor. Moreover, this reduction in N digestibility gradually returned to normal during the course of the experiment which Sykes and Coop (1977) postulated could be due to an increase in intestinal digestion of protein.

A more likely explanation for a protein deficiency is the increased leakage of plasma proteins into the digestive tract through parasitised mucosae (Dargie, 1980). Field and Coop (personal communication) have shown that sloughing of mucosal cells is a greater source of N loss into the lumen of the gut. Dargie (1980) postulated that there was a preferential channelling of amino acids into immunoglobulin synthesis and also that the increased protein synthesis necessary to replace the enhanced losses of

protein into the gut of parasitised sheep could cause deficiency in other tissues and reduce the synthesis in bone matrix.

An alternative hypothesis is that ostertagiasis leads to a reduction in plasma thyroxine levels (Sykes et al, 1977). Thyroxine is an important hormone in the control of growth and it is thought that thyroxine somehow promotes the effects of growth hormone on protein synthesis.

Bone is a living tissue consisting of a protein matrix, upon which Ca salts are deposited. The cells responsible for laying down this matrix are osteoblasts. Growth of a long bone depends upon actively proliferating layers of cartilage (epiphyseal plates) at the ends of the bone. The osteoblasts at the edge of the epiphyseal plates convert the cartilaginous tissue into bone while new cartilage is simultaneously formed in the plates. Growth hormone, another important hormone in the control of growth, promotes this lengthening by stimulating protein synthesis in both the cartilaginous centre and bony edge of the epiphyseal plates as well as by increasing the rate of osteoblast mitosis.

The finding in relation to Ca is not definitive as the diet used contained more Ca than required and under these circumstances Ca is absorbed according to needs (ARC, 1980) and consequently retention and absorption are completely confounded. The observation that the endogenous faecal excretion of Ca of the OC animals was elevated could mean that the efficiency of absorption of endogenously secreted

Ca was reduced or that the action of the worms increased the leakage of Ca through the gastric mucosa.

The findings of this work are the first direct evidence that T. colubriformis reduces the efficiency of absorption of dietary P. Other workers have reported a reduction in the apparent absorption of P in T. colubriformis infections (Reveron et al, 1974a; Sykes and Coop, 1976b), but apparent absorption measures retention in growing lambs and not true absorption of P (Lofgreen, 1960). The fall in the efficiency of absorption of dietary P due to the action of intestinal parasitism appeared to be about 30% and this best estimate was based on a comparison between the data for the TC and the combined C and OC groups.

The lower plasma concentrations of P in the TC group suggest that the parasites induced a P deficiency through their effect on P absorption, despite their effect on the skeleton reducing P requirement (Sykes and Coop, 1976b). However, there is some evidence which suggests that this may not be the complete explanation; one of the C animals on the lower P intake had the same P status as TC animals on the higher P intake, yet had a normal plasma P concentration. A possible explanation for this finding is that, unlike normal animals, T. colubriformis infected sheep lose significant quantities of endogenous P not only in the saliva but also in the small intestine. These losses could be the result of the parasite increasing the rate of shedding of mucosal cells, intestinal secretions and loss of body fluid. Furthermore, such losses would be outwith the animals control and together with the minimum

loss of P in saliva could lead to a P deficiency. The flow of endogenous P at the abomasum of TC animals was less than half that of the C or OC animals showing that salivary P secretion, the main component of endogenous P losses into the stomach region, was greatly reduced in T. colubriformis infections.

An increased loss of endogenous P in the small intestine of TC animals would lead to an apparently greater reduction in the efficiency of absorption of endogenous than exogenous P in the small intestine. A small effect can be seen in the data in Table 4.6, but a greater one if more simplified and hence more accurate methods are used to measure absorption. These are based on the observation that no appreciable absorption of P occurred in the stomach region and that absorption of exogenous P was confined to the small intestine. Thus endogenous P secretion at the abomasum is the total flow of P less the P intake and the overall absorption of exogenous P is equal to that in the small intestine. The efficiency of absorption of endogenous P in the small intestine by the C and OC animals was 0.73 whereas the mean value for the TC animals was only 0.082, the low value being due to two animals showing net secretion of P in the small intestine. Since the comparable figures for exogenous P were 0.59 and 0.38, this is clear evidence that appreciable losses of endogenous P occurred in the small intestine. There was no evidence that infection with O. circumcincta increased endogenous P losses in the abomasum.

The endogenous faecal excretion of Ca was elevated in the TC group. Since there was no evidence to indicate that T. colubriformis affected Ca absorption in the small

intestine under the conditions of the experiment, the higher Ca endogenous secretion must have originated from a leakage of body fluid into the small intestine. The Ca could not have originated from the mucosal cells because of the low concentration of Ca in cells.

Although at least part of the poor bone mineralisation recorded in animals infected with T. colubriformis can be attributed to the P deficiency induced by the action of the parasites, it must not be forgotten that the metabolic processes which cause poor mineralisation in O. circumcincta infections also probably operate in intestinal parasitism. Dargie (1980) and others have argued that the increased protein synthesis necessary to replace the enhanced losses of protein into the gut of parasitised sheep could lead to deficiency in other tissues and reduce the synthesis in bone matrix. Support for the sensitivity of the skeleton to nutrition arises from the observation that bone mineralisation stops in young sheep on the hill in winter despite the fact that their skeletons are only half mineralised and that the dietary intake of Ca and P appears adequate (Field and Harris, personal communication). Recent comparative studies have shown that the flow of exogenous N into the gut of animals infected with T. colubriformis and O. circumcincta are similar (Field and Coop, personal communication). Poppi et al (1981) have shown that the increase in endogenous N entering the small intestine of sheep infected with T. colubriformis was not only due to plasma protein leakage but must also have come from other sources, such as cell sloughing and

mucin secretions. It is thus possible that the greater effect of T. colubriformis on bone mineralisation is caused by the combined N and P effect, whereas with O. circumcincta only the protein effect may be operating. Evidence for this view comes from the calculation that a P intake of 3 g/d would meet the endogenous P requirements of the TC group, yet poor bone mineralisation was seen in the infected sheep of Sykes and Coop (1976b) given 10.2 g P/day.

The changes with time in faecal egg counts for both the TC and OC groups showed no obvious trend, presumably due to the short duration of the experiment. Sykes and Coop (1976b; 1977) found that maximum concentration of eggs in the faeces was reached after eight and nine weeks for the TC and OC animals respectively.

Faecal egg counts are recognised as an insensitive quantitative measure of pathogenicity of parasitic infections. Thomas and Waller (1975), on the basis of a significant correlation between Ostertagia burdens and plasma pepsinogen concentrations in grazing lambs, considered the latter to be a useful diagnostic tool in ostertagiasis. Due to the small number of animals in the OC group no correlation was found between plasma pepsinogen and abomasal worm burdens in this work.

The high flow rate at the abomasum of the OC animals probably arose from increased secretion by the glands in the gastric mucosa; Coop et al (1977) have described hypertrophy of the upper fundic mucosa leading to a large increase in the

number of mucosal secreting cells in Ostertagia infections. The additional flow of Na was consistent with increased glandular secretion and with the finding of higher concentrations of Na in the abomasal fluid from calves infected with Ostertagia ostertagi (Jennings et al, 1966). The large increase in flow rate is not consistent with the known losses of body fluids in ostertagiasis (40-130 ml per day, Holmes and MacLean, 1971), nor was there any evidence for a large increase in the flow of OM which would be necessary if appreciable losses of body fluid had occurred.

Recently attention has been turning to the role of the endocrine system in the control of P absorption from the digestive tract and it is now generally accepted that mammals fed diets low in P adapt via a humoral mechanism so that the efficiency of the process by which phosphate (and also Ca) is absorbed from the small intestine is increased (Fox and Care, 1978; Fox et al, 1978). The common mechanism for the adaptive change to a diet low in either P or Ca is thought to involve an increased production of 1,25 - $(OH)_2$ D_3 in the kidney.

It is well established using rats (Haussler et al, 1976) and pigs (Sommerville et al, 1980) that hypophosphataemia, induced by a low P diet, leads to an increase in the circulating level of 1,25 - $(OH)_2$ D_3. The results of Braithwaite (1981) suggest that P absorption is enhanced directly by 1α - OH - D_3 which is a synthetic analogue of Vitamin D. However, in animals infected with intestinal parasites there is a reduction in P absorption. Presumably

the physiological effect of the intestinal parasites on the site of P absorption is such that the enhancing effect of 1,25 - $(OH)_2$ D_3 is insufficient to overcome the parasitic effect.

In view of the important role which 1,25 - $(OH)_2$ D_3 plays in maintaining Ca and P homeostasis, it would be worthwhile undertaking experiments in order to establish its role in abomasal and intestinal parasitism.

In view of the speculation that the osteoporosis associated with chronic Ostertagia circumcincta is a matrix osteoporosis resulting from an energy or protein deficiency, the concentrations of corticosteroids, insulin and thyroxine hormones, which are of major importance in the control of protein metabolism, should be the topics for further investigations. If more was known about the relative effectiveness with which the skeleton and soft tissues compete for nutrients it might be possible to decide which is the limiting nutrient.

The design of experiments for future studies should include a) the infusion of P salts in order to supply adequate P for bone mineralisation and therefore eliminate P as the limiting nutrient; at the same time bone growth could be monitored by the use of tetracycline, a marker which adsorbs onto the bone surface, b) the infusion of hydrochloric acid to maintain the abomasal pH and therefore find out what effect, if any, this has on protein digestion and c) protein turnover studies.

Finally, by directing future studies towards measurement of mineral absorption at the gut level together with its rate of accretion into and resorption from bone using compartmental analysis (Braithwaite et al, 1969), it should be possible to construct a more accurate picture of the dynamics of the processes involved in bone formation.

TABLE 4.1 Details of the three balance trials in Series II

BALANCE TRIAL	TREATMENT	SHEEP NO	INTAKE (kg/d)	DURATION OF TRIAL: PRELIMINARY PERIOD	DURATION OF TRIAL: COLLECTION PERIOD	IV DOSE (mCi): ^{32}P	IV DOSE (mCi): ^{45}Ca	IR DOSE (μCi/d): ^{51}Cr	IR DOSE (μCi/d): ^{103}Ru
A	C	757 762 751 763	1	7	8	2	0.27	50	10
	OC	754 722 800 841	1	7	8	2	0.27	50	10
B	TC	759 838 828	1	7	8	1.5	0.27	50	10
	TC	830 = 808 765	0.7	7	8	1.5	0.27	50	10
	OC	852	0.7	7	8	1.5	0.27	50	10
C	C	757 762 751 763	0.7	7	8	1	0.27	50	10

IV = intravenous IR = intraruminal = force fed to different degrees

TABLE 4.2 The total number of worms and the pathological findings in the animals which were killed at the end of the experiment (Series II)

ANIMAL NO	SEX	TOTAL WORM POPN.	LARVAL INPUT	% RECOVERY	OC LESION IN ABOMASUM	TC LESION IN S INTESTINE
708 C	F	-	-	-	NIL	NIL
774 C	F	-	-	-	NIL	NIL
777 C	M	-	-	-	NIL	NIL
759 TC	M	51800	144000	36.0	NIL	VERY SEVERE
765 TC	M	8600	144000	6.0	NIL	SEVERE
775 TC	F	19000	144000	13.2	NIL	SEVERE
808 TC	F	15000	144000	10.4	NIL	VERY SEVERE
813 TC	M	34200	144000	23.8	NIL	VERY SEVERE
828 TC	M	14400	144000	10.0	NIL	VERY SEVERE
830 TC	F	11800	144000	8.2	NIL	VERY SEVERE
838 TC	F	24200	144000	16.8	NIL	VERY SEVERE
722 OC	M	48600	240000	20.3	SEVERE	NIL
754 OC	F	15300	240000	6.4	SEVERE	NIL
764 OC	M	27900	240000	11.6	SEVERE	NIL
800 OC	M	12900	240000	5.4	SEVERE	NIL
841 OC	F	37500	240000	15.6	SEVERE	NIL
842 OC	F	11700	240000	4.9	SEVERE	NIL
852 OC	M	55900	240000	23.3	SEVERE	NIL
854 OC	F	28100	240000	11.7	SEVERE	NIL

TABLE 4.3 Intake and flow rates of dry matter (DM), organic matter (OM) and sodium (Na) along the digestive tract of the lambs in groups C, TC and OC (Series II)

TREATMENT	C		TC		OC		SD
FOOD INTAKE (g/d)	1000	700	1000	700	1000	700	
n	4	4	2	3	3	1	
LIVEWEIGHT (kg)	46	47	47	40	42	36	
DM INTAKE (kg/d)	0.86	0.60	0.82	0.60	0.82	0.60	
OM INTAKE (kg/d)	0.77	0.54	0.73	0.51	0.73	0.54	
ABOMASAL FLOW (kg/d)							
LIQUID (WATER)(l/d)	8.34	6.26	9.45	5.98	17.9	15.6	1.12
TOTAL FLOW OF DM	0.62	0.45	0.54	0.36	0.69	0.53	0.0445
TOTAL FLOW OF OM	0.53	0.37	0.44	0.30	0.54	0.38	0.0386
Na (g/d)	11.6	9.96	19.0	11.5	36.1	33.6	2.53
ILEAL FLOW (kg/d)							
LIQUID (l/d)	4.58	2.99	4.96	3.24	4.98	2.13	0.330
TOTAL FLOW OF DM	0.40	0.34	0.38	0.26	0.34	0.22	0.0451
TOTAL FLOW OF OM	0.32	0.25	0.30	0.20	0.26	0.18	0.0421
Na (g/d)	14.4	10.0	18.0	13.1	13.8	7.4	3.55
FAECAL (kg/d)							
DM	0.32	0.21	0.27	0.20	0.29	0.19	0.0216
OM	0.28	0.18	0.22	0.16	0.25	0.16	0.0198

TABLE 4.4a

Digestibility of DM for each animal which ate 1000g/day (Series II)

SHEEP NO	LIVE-WEIGHT (kg)	FEED INTAKE (g/d)	DM INTAKE (g/d)	FAECAL OUTPUT FRESH WT (g/d)	FAECAL OUTPUT DRY WT (g/d)	DIGESTIBILITY OF DM
757 C	46.25	1000	862	775	323	0.63
762 C	46.5	1000	862	689	293	0.66
751 C	44.0	1000	862	857	356	0.59
763 C	43.5	1000	862	895	327	0.62
828 TC	48.0	1000	861	668	290	0.66
759 TC	43.75	901	776	578	248	0.68
754 OC	45.75	1000	862	712	303	0.65
800 OC	40.0	956	824	696	314	0.62
722 OC	41.75	895	772	813	267	0.65

TABLE 4.4b Digestibility of DM for each animal which ate 700g/day (Series II)

SHEEP NO	LIVE-WEIGHT (kg)	FEED INTAKE (g/d)	DM INTAKE (g/d)	FAECAL OUTPUT FRESH WT (g/d)	FAECAL OUTPUT DRY WT (g/d)	DIGESTIBILITY OF DM
757 C	47.5	700	601	493	207	0.66
762 C	45.5	700	601	530	233	0.61
751 C	43.0	700	601	416	191	0.68
763 C	42.5	700	601	526	205	0.66
830 TC	36.75	700	603	609	203	0.66
808 TC	40.25	700	603	447	198	0.67
765 TC	45.5	666	573	427	178	0.69
838 TC	36.5	612	527	498	212	0.60
852 OC	36.25	700	603	406	192	0.68

TABLE 4.5a Digestibility of OM for each animal which ate 1000g/day (Series II)

SHEEP NO	LIVE-WEIGHT (kg)	FEED INTAKE (g/d)	OM INTAKE (g/d)	FAECAL OUTPUT FRESH WT. (g/d)	FAECAL OUTPUT ORG. MATTER (g/d)	DIGESTIBILITY OF ORGANIC MATTER
757 C	46.25	1000	771	775	274	0.64
762 C	46.50	1000	771	689	247	0.68
751 C	44.0	1000	771	857	310	0.60
763 C	43.5	1000	771	895	280	0.64
828 TC	48.0	1000	767	668	242	0.68
759 TC	43.75	901	691	578	206	0.70
754 OC	45.75	1000	771	712	255	0.67
800 OC	40.0	956	737	696	267	0.64
722 OC	41.75	895	690	813	226	0.67

TABLE 4.5b Digestibility of OM for each animal which ate 700g/day (Series II)

SHEEP NO	LIVE-WEIGHT (kg)	FOOD INTAKE (g/d)	OM INTAKE (g/d)	FAECAL OUTPUT FRESH WT. (g/d)	FAECAL OUTPUT ORG. MATTER (g/d)	DIGESTIBILITY OF ORGANIC MATTER
757 C	47.5	700	540	493	175	0.68
762 C	45.5	700	540	530	197	0.64
751 C	43.0	700	540	416	165	0.69
763 C	42.5	700	540	526	179	0.67
830 TC	36.75	700	537	609	171	0.68
808 TC	40.25	700	537	447	163	0.70
765 TC	45.5	666	511	427	145	0.72
838 TC	36.5	612	470	498	179	0.62
852 CC	36.25	700	537	406	162	0.70

TABLE 4.6 The flows (g/d) and efficiency of absorption of endogenous and exogenous phosphorus along the digestive tract of the lambs (Series II)

TREATMENT	n	INTAKE (g/d)	URINE (g/d)	ABOMASUM END (g/d)	ABOMASUM EXO (g/d)	ILEUM END (g/d)	ILEUM EXO (g/d)	FAECES END (g/d)	FAECES EXO (g/d)	EFFICIENCY OF ABSORPTION: SMALL INTESTINE END	SMALL INTESTINE EXO	OVERALL END	OVERALL EXO	PLASMA CONCN mg/l
C	4	5.71	0.90	6.25	6.19	2.02	2.18	1.53	2.30	0.68	0.65	0.76	0.60	64.9
	4	3.92	0.91	6.21	4.49	1.40	1.55	0.89	1.55	0.77	0.65	0.84	0.65	75.0
TC	2	5.83	0.02	3.27	5.66	2.21	3.87	1.32	3.54	0.32	0.32	0.60	0.39	35.1
	3	4.22	0.03	2.19	3.74	1.63	2.90	1.63	2.58	0.25	0.22	0.25	0.39	34.0
OC	3	5.45	0.35	6.44	5.35	2.18	2.33	1.84	2.73	0.66	0.56	0.71	0.50	61.5
	1	4.22	0.13	6.58	3.24	1.66	1.11	1.27	1.69	0.75	0.66	0.81	0.60	89.8
SD				1.17	0.607	0.587	0.686	0.351	0.582	0.112	0.126	0.065	0.123	

TABLE 4.7 The flow of salivary P (g/d) in the C, TC and OC groups (Series II)

TREATMENT	n	INTAKE (g/d)	RUMEN TOTAL FLOW OF P (g/d)	SALIVARY P (g/d)
C	4	5.71	12.25	6.54
	4	3.92	9.77	5.85
TC	2	5.83	6.16	0.33
	4	4.22	5.44	1.22
OC	3	5.45	12.10	6.65
	1	4.22	8.25	4.03

TABLE 4.8 Flow, absorption and endogenous faecal loss of calcium in the digestive tract (Series II)

TREATMENT	n	INTAKE (g/d)	FLOW		FAECES		OVERALL EFFICIENCY OF ABSORPTION	PLASMA CONCENTRATION (mg/l)
			ABOMASUM (g/d)	ILEUM (g/d)	ENDOGENOUS (g/d)	EXOGENOUS (g/d)		
C	4	8.04	8.15	7.55	0.57	6.57	0.18	87.0
	4	5.28	6.06	5.61	0.37	4.11	0.22	83.5
TC	2	8.30	8.00	8.05	0.77	5.95	0.28	89.1
	3	6.00	5.28	6.10	0.78	4.34	0.27	89.6
OC	3	7.67	9.50	7.27	0.74	6.49	0.16	85.2
	1	6.01	6.59	4.11	0.65	3.65	0.39	77.3
SD			0.806	1.06	0.0805	0.507	0.0582	

TABLE 4.9 The abomasal pH values at intervals during the experiment (Series II) for animals in the C, TC and OC groups

TREATMENT	SHEEP NO	DAY 24	DAY 25	DAY 26	DAY 27	DAY 28	DAY 29	DAY 30
C	757	2.60	2.70	2.80	2.50	2.60	2.70	2.60
C	762	3.10	2.90	2.80	2.90	2.70	2.90	2.70
C	751	2.60	2.50	2.50	2.50	2.50	2.50	2.50
C	763	2.90	2.90	2.60	2.70	2.60	2.40	2.40
OC	841	6.00	5.90	6.00	6.20	6.40	6.50	6.50
OC	722	2.80	2.90	2.80	2.70	2.90	2.90	3.00
OC	800	4.30	3.30	3.00	3.30	3.40	3.20	3.10
OC	754	5.10	5.00	4.70	4.60	4.20	4.20	3.70
		DAY 37	DAY 38	DAY 39	DAY 40	DAY 41	DAY 42	DAY 43
TC	765	3.10	3.50	3.00	3.40	3.50	3.40	3.20
TC	830	2.80	2.60	2.60	2.50	2.20	2.60	2.40
TC	808	2.70	2.70	2.70	2.50	2.40	2.60	2.50
TC	759	2.90	2.90	2.80	2.80	2.60	2.70	2.70
TC	838	2.70	2.70	2.60	2.10	2.60	2.20	2.00
TC	828	2.90	2.90	2.80	2.90	2.40	2.40	3.10

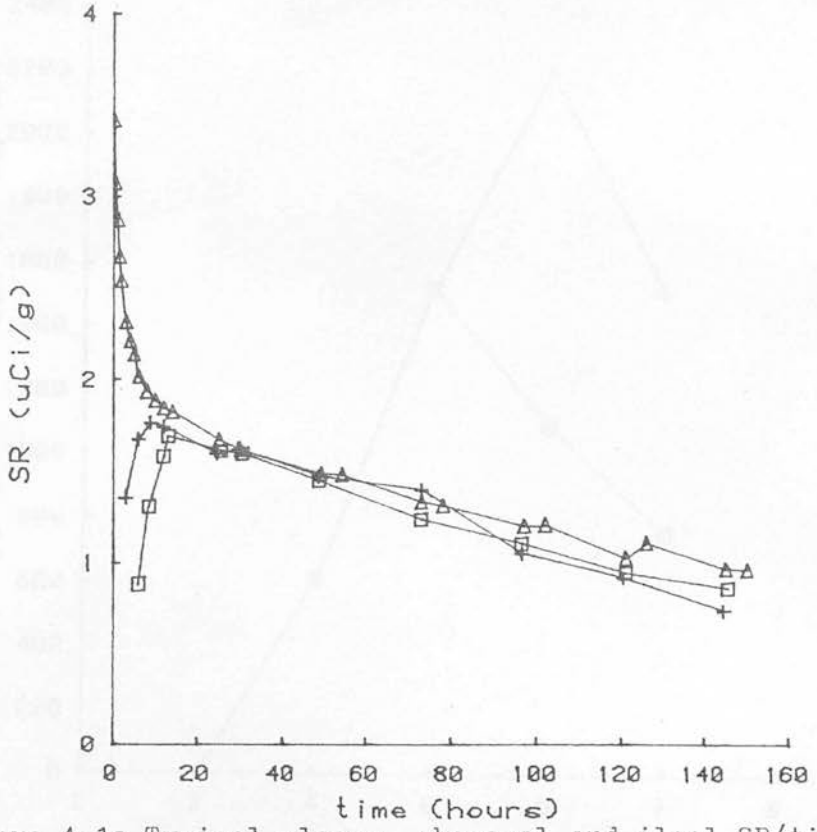

Figure 4.1a Typical plasma, abomasal and ileal SR/time curves for P (series II).
△-△ Plasma +-+ Abomasum □-□ Ileum

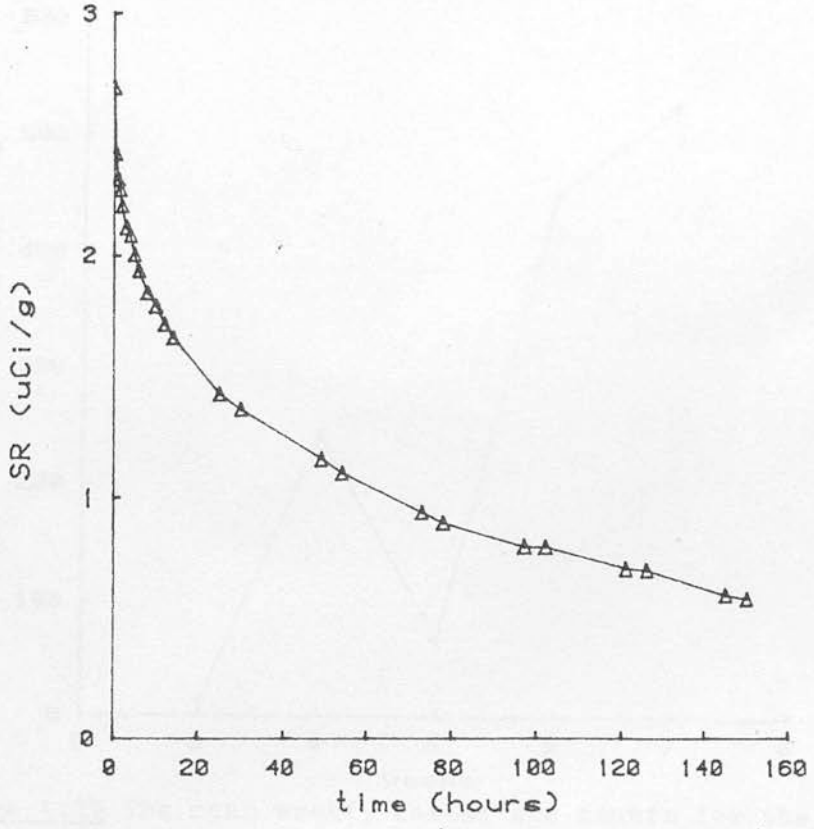

Figure 4.1b Typical plasma SR/time curve for Ca (series II).

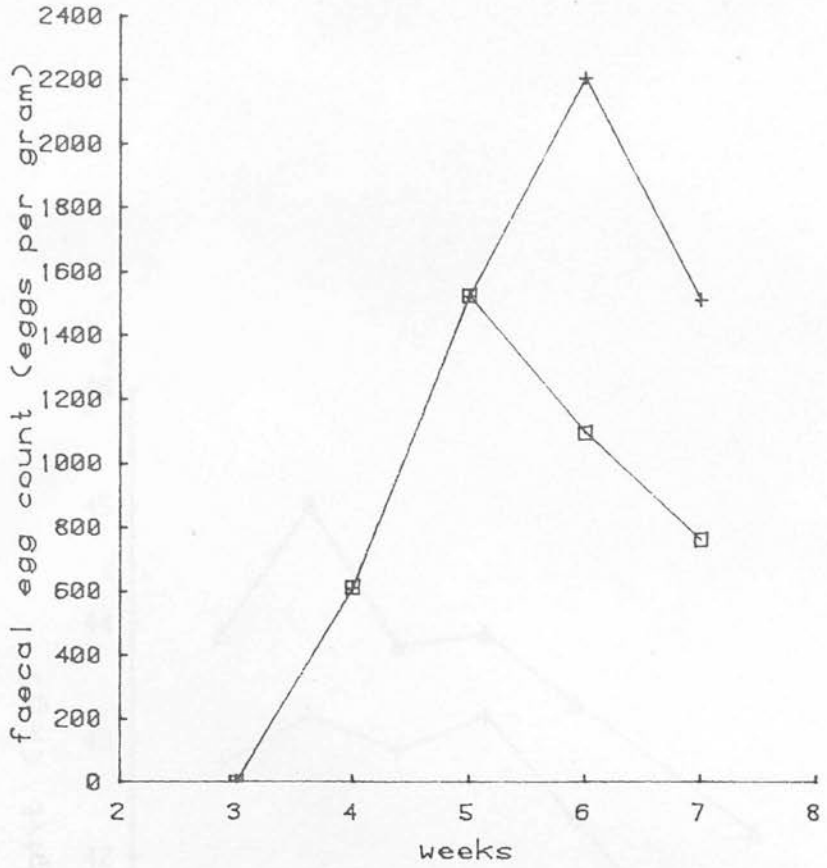

Figure 4.2a The mean weekly faecal egg counts for the TC group (series II).
□-□ If exclude animal no. 813 as it had a very high egg count.

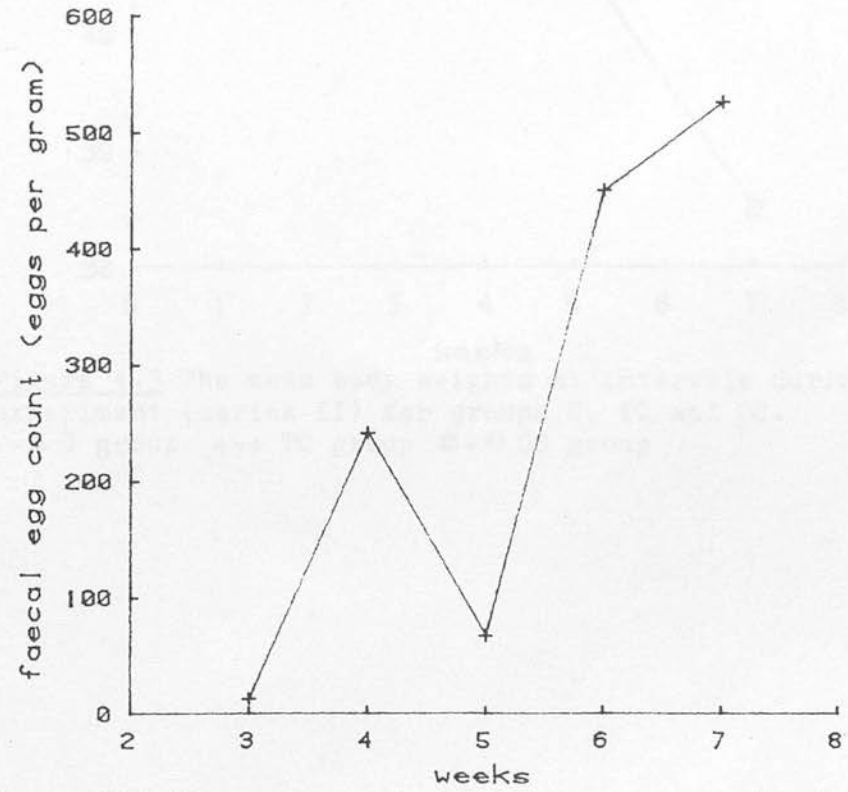

Figure 4.2b The mean weekly faecal egg counts for the OC group (series II).

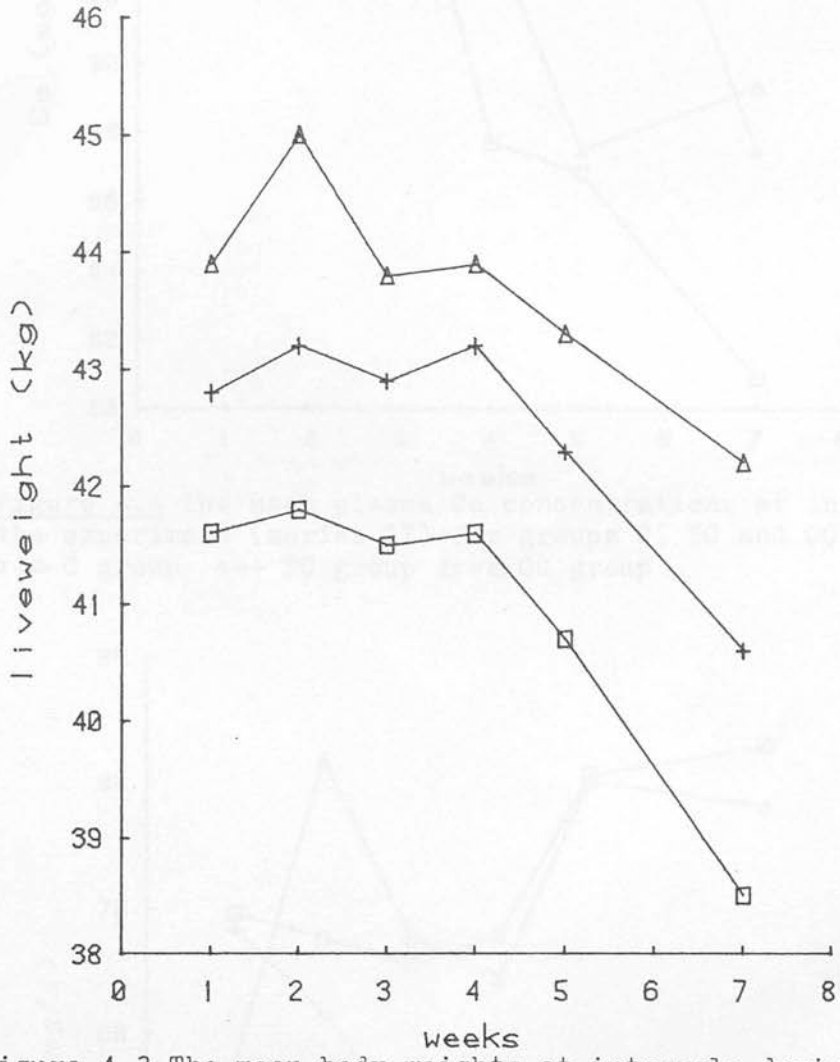

Figure 4.3 The mean body weights at intervals during the experiment (series II) for groups C, TC and OC.
△-△ C group +-+ TC group □-□ OC group

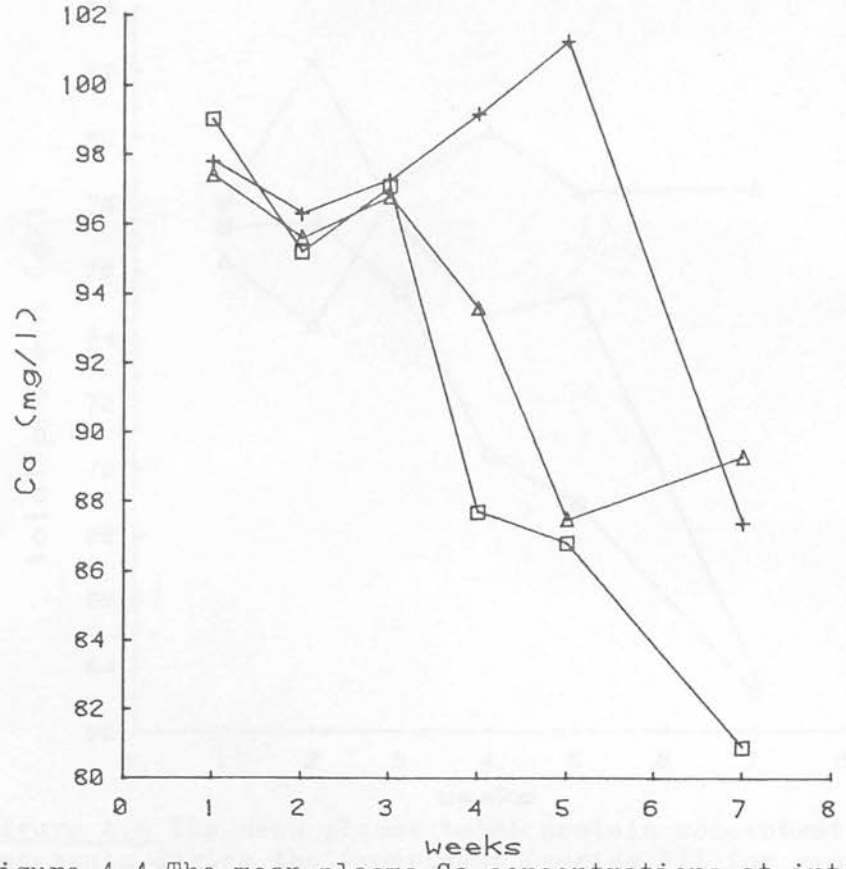

Figure 4.4 The mean plasma Ca concentrations at intervals during the experiment (series II) for groups C, TC and OC.
△-△ C group +-+ TC group □-□ OC group

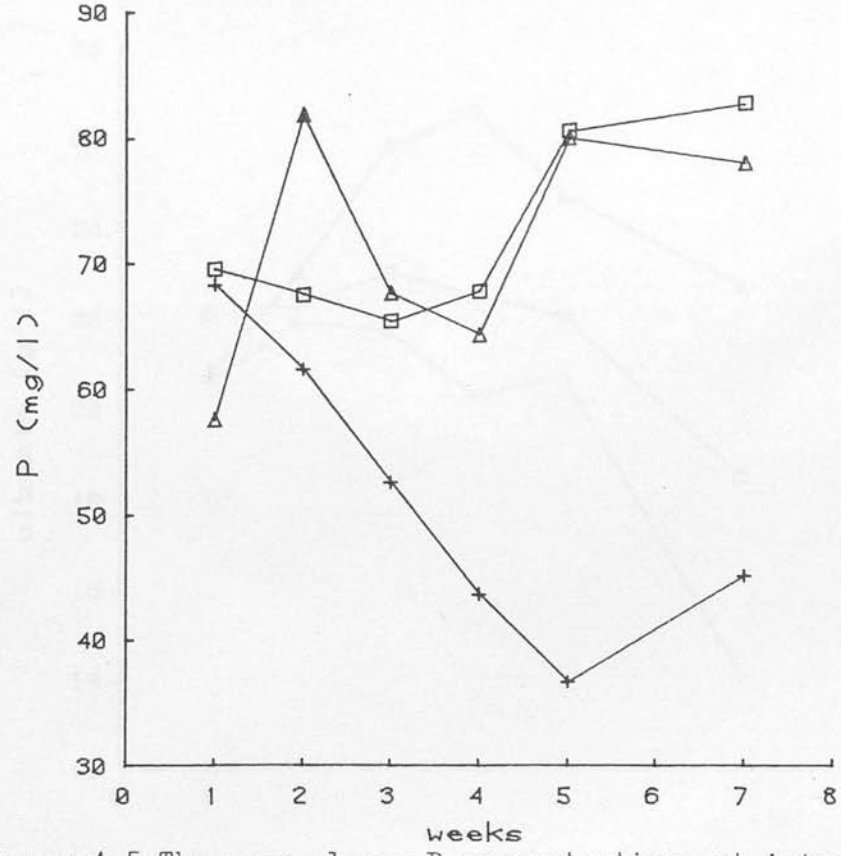

Figure 4.5 The mean plasma P concentrations at intervals during the experiment (series II) for groups C, TC and OC.
△-△ C group +-+ TC group □-□ OC group

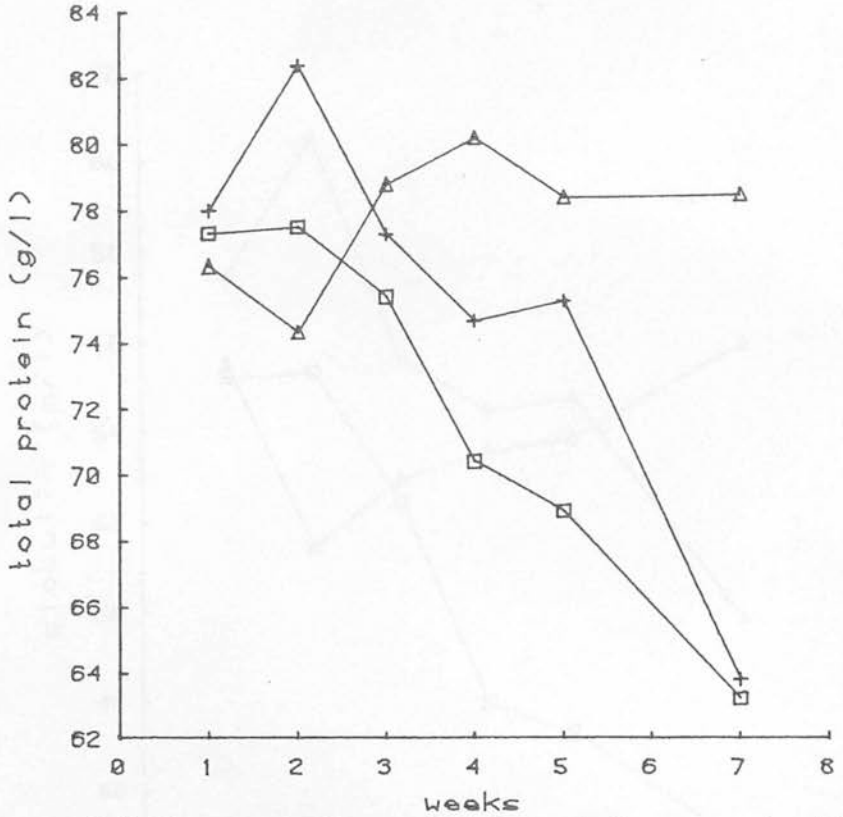

Figure 4.6 The mean plasma total protein concentrations at intervals during the experiment (series II) for groups C, TC and OC.
△-△ C group +-+ TC group □-□ OC group

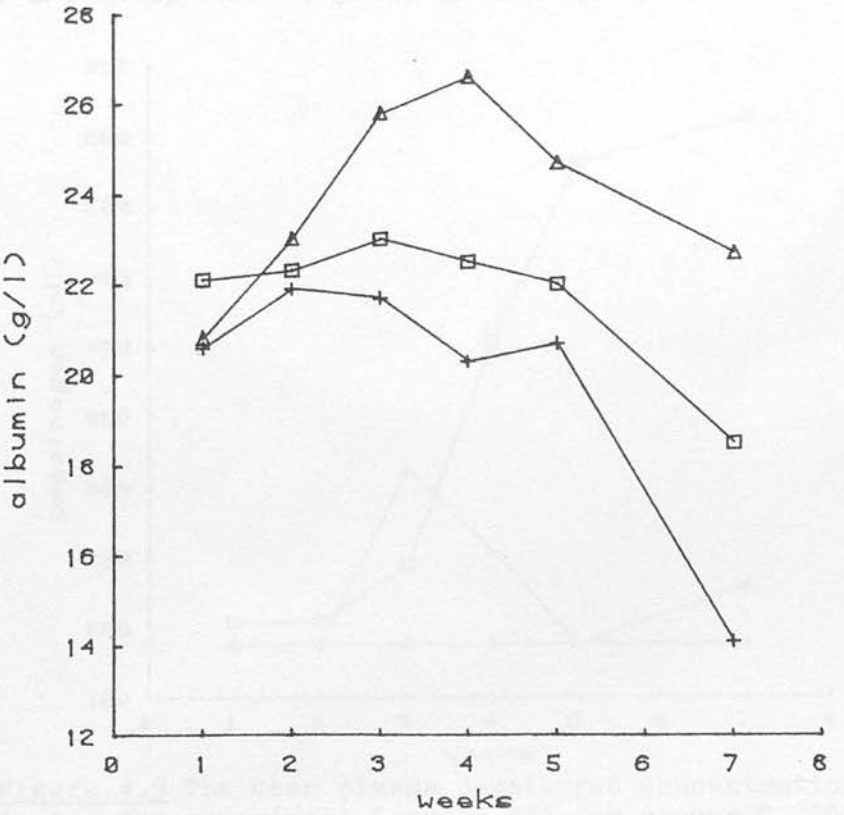

Figure 4.7 The mean plasma albumin concentrations at intervals during the experiment (series II) for groups C, TC and OC.
△-△ C group +-+ TC group □-□ OC group

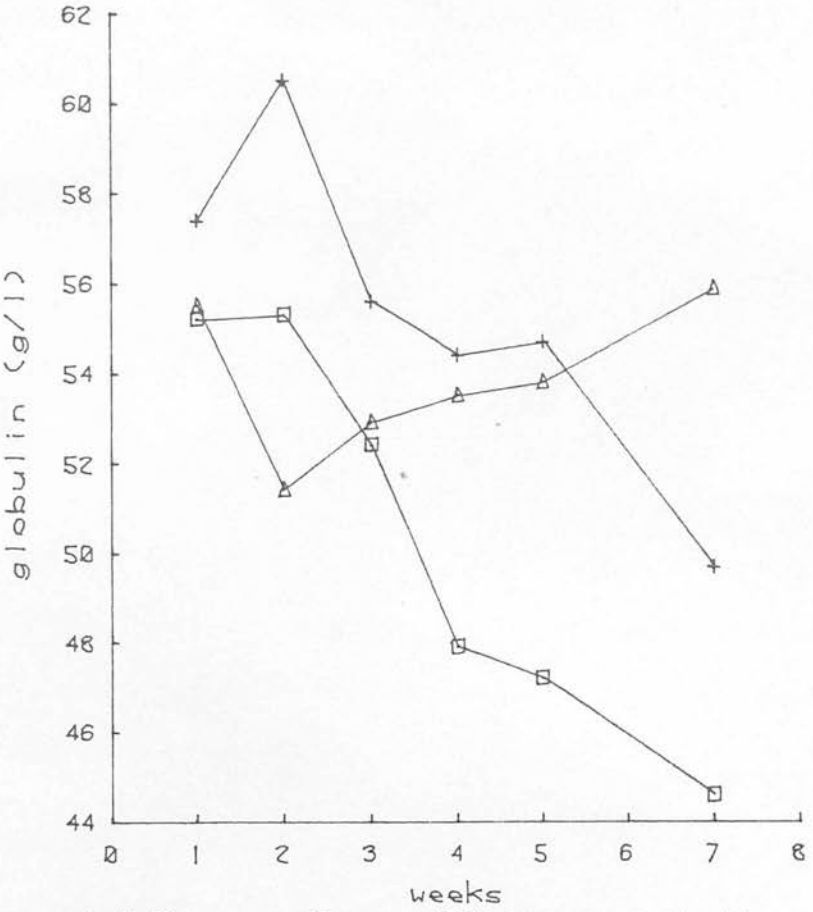

Figure 4.8 The mean plasma globulin concentrations at intervals during the experiment (series II) for groups C, TC and OC.
△-△ C group +-+ TC group □-□ OC group

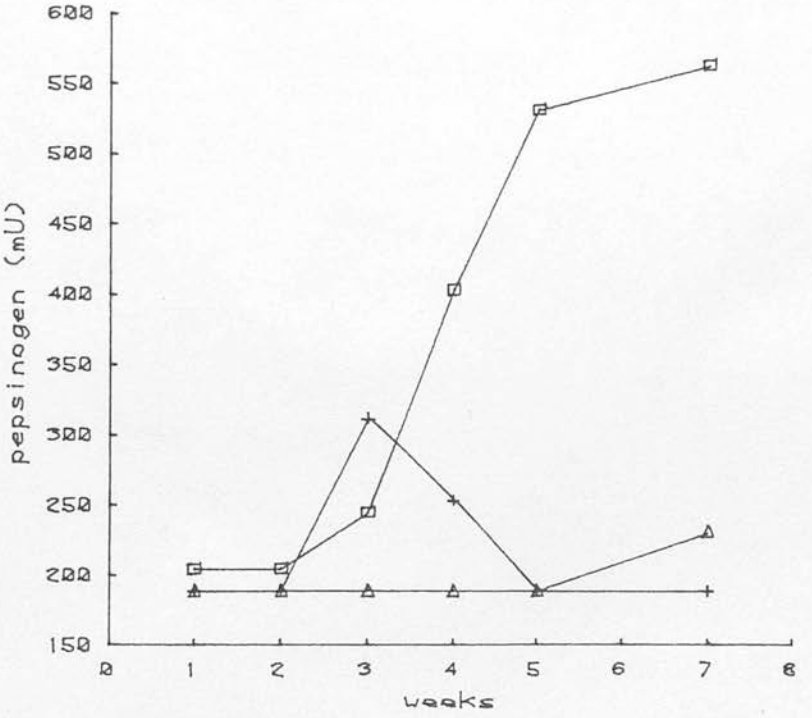

Figure 4.9 The mean plasma pepsinogen concentrations at intervals during the experiment (series II) for groups C, TC and OC.
△-△ C group +-+ TC group □-□ OC group

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DECLARATION

I declare that this thesis has been composed by myself and that subject to the above acknowledgements, the work described here is my own.

Wilma D. Wilson

22/1/82.

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APPENDIX 2.1 Composition of Ruminant A diet as given by Wainman, Blaxter and Pullar (1970)

FORMULATION *	%	CHEMICAL COMPOSITION OF DRY MATTER (%)	
Chopped Barley Straw	40.00	CRUDE PROTEIN	13.5
Extracted Groundnut Meal	7.00	CRUDE FAT	2.5
Barley	12.50	CRUDE FIBRE	19.0
Wheatfeed	23.25		
Molasses	10.00		
Urea	1.00		
Tallow	1.25		
Salt	1.50		
Dicalcium Phosphate	1.50		
Sodium Bicarbonate	2.00		

* With the addition of vitamins A, D3 and E, trace elements and iodide

APPENDIX 2.2 Typical computer print-out from an automated chemical assay

The computer programme consisted of two parts :

a quality control section and a results section

Quality Control

Experiment number

First lab. number

Number of peaks

Versatol readings before drift (the x co-ordinates from the pencil follower)

Versatol readings after drift

Regression coefficient and residual variance
(of the best line in a graph of the x co-ordinates of the standards v the concentrations of the standards)

Uncorrected Versatol concentrations (initial and final)

Actual Versatol concentrations (as given by manufacturers)

Versatol multiplication factor

Actual and estimated concentrations of standards

Results

Pool plasma concentrations

Mean and S.D. of pool plasma concentrations

Drift-corrected Versatol concentrations (initial and final)

Sample concentrations, 1-64

APPENDIX 3.1 The egg counts (eggs/gram fresh faeces) for each animal which completed Series I at intervals during the experiment

ANIMAL No.	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9	WEEK 10	WEEK 11	WEEK 12	WEEK 13	WEEK 14
440 OC	4	284	651	242	1827	879	84	415	1290	0	160	0
295 OC	7	84	851	557	1817	399	116	0	0	7	0	21
427 OC	46	116	746	294	1628	42	42	378	110	35	0	10
352 OC	4	200	326	126	176	809	32	360	360	10	0	10

APPENDIX 3.2 The body weights (kg) of each animal which completed Series I at intervals during the experiment

ANIMAL NO	WEEK 5	WEEK 7	WEEK 9	WEEK 11	WEEK 14
362 C	32	33	34	BT	38
394 C	27	BT	27	28	32
339 C	28	BT	26	30	30.5
431 C	29	30	31.5	32.5	33.5
309 C	26	25	26.5	26.5	27
340 C	35	36	37.5	BT	38.5
441 TC	36	34	33	BT	33
423 TC	34	35	33	BT	35
350 TC	25	23.5	23.5	24	26
440 OC	35	36	34.5	BT	32.5
295 OC	22	21	22	23.5	25
427 OC	39	40.5	38.5	BT	42
352 OC	34	-	34.5	BT	34.5

BT = Balance Trial

APPENDIX 3.3 The mean daily food intake (g/d) for each animal which completed Series I

ANIMAL NO	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9	WEEK 10	WEEK 11	WEEK 12	WEEK 13
362 C	1200	1200	1200	1200	1200	1200	1169	1200	1192
394 C	984	797	651	724	1129	1194	1154	1118	1174
339 C	1086	796	793	738	1143	1200	1200	1159	1188
431 C	1200	1200	1200	1080	1200	1186	1169	1200	1188
309 C	1200	1178	1066	995	1142	1176	1088	903	838
340 C	1200	1200	1200	1200	1200	1200	1167	1200	1180
441 TC	1200	1200	1200	1200	1200	1200	1194	1071	1148
423 TC	1200	1200	1139	1164	1200	1153	1152	1200	1142
350 TC	817	789	851	753	835	919	868	905	980
440 OC	1200	1200	1079	734	916	574	512	642	910
295 OC	831	921	802	829	1032	906	954	838	929
427 OC	1200	1200	1200	1200	1200	1200	842	1122	1161
352 OC	974	1168	1200	1170	1031	1091	1106	1151	1174

APPENDIX 3.4 Phosphorus: Changes in SR(μCi/g) with time after dosing in faeces for the individual animals in Balance Trial A (Series I)

	339 C	394 C	425 TC	307 TC	301 OC	422 OC
DAY 1	47.839	29.134	21.891	42.511	33.552	31.582
DAY 2	99.686	86.304	68.078	90.016	76.394	84.578
DAY 3	49.583	52.688	50.170	44.703	47.102	42.706
DAY 4	28.382	31.407	32.203	25.796	35.395	31.026
DAY 5	22.335	21.968	25.650	15.645	26.089	19.133
DAY 6	15.600	16.823	27.294	11.238	18.927	16.153
DAY 7	13.394	15.787	20.329	10.660	14.466	13.663
DAY 8	11.063	15.052	14.657	10.797	11.628	11.450
DAY 9	9.587	12.519	10.982	8.557	6.542	6.214
DAY 10	6.790	10.030	7.620	6.260	3.060	2.140
DAY 11	3.130	7.140	5.870	6.300	5.770	-

APPENDIX 3.5 Phosphorus: Changes in SR (μCi/g) with time after dosing in urine for the individual animals in Balance Trial A (Series I)

	339 C	394 C	425 TC	307 TC	301 OC	422 OC
DAY 1	59.749	63.305	12.358	11.880	190.292	175.601
DAY 2	51.127	60.905	10.947	6.185	55.079	63.995
DAY 3	35.218	43.360	8.118	13.671	46.707	43.871
DAY 4	38.656	33.266	8.101	18.258	35.333	34.071
DAY 5	29.452	21.903	3.374	12.792	24.811	21.838
DAY 6	27.698	18.640	13.638	15.216	18.823	18.335
DAY 7	23.173	16.933	10.820	14.410	13.303	15.621
DAY 8	19.878	15.352	9.311	14.023	12.802	13.887
DAY 9	16.667	14.729	6.975	11.838	11.169	12.092
DAY 10	15.436	12.584	4.874	12.204	9.629	11.890
DAY 11	12.038	12.144	5.298	11.290	7.906	-

APPENDIX 3.6

Plasma pepsinogen concentrations (mU l^{-1} min^{-1}) for the 12 animals used in the pepsinogen study (Series 1)

ANIMAL NO	DAY 1	DAY 5	DAY 9	DAY 13	DAY 17	DAY 21	DAY 25	DAY 33	DAY 41	DAY 49	DAY 57
362 C	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200
353 C	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200
340 C	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200
291 C	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200
383 C	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200
339 C	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200	< 200
427 OC	487	562	637	637	937	937	1161	1161	1292	1573	1854
440 OC	487	562	637	562	730	768	730	1161	1292	1573	2247
352 OC	487	487	562	562	730	730	730	730	861	981	918
387 OC	< 200	< 200	< 200	487	730	730	730	730	861	861	1086
301 OC	562	562	562	562	730	730	768	730	1048	1381	1685
312 OC	562	562	562	562	1161	1161	1161	1161	1292	1573	1873

APPENDIX 4.1

The egg counts (eggs/gram fresh faeces) for each animal at intervals during the experiment (Series II)

ANIMAL NO	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7
759 TC	0	620	1712	1040	977
765 TC	0	462	2000	1638	1344
775 TC	0	998	1900	1449	714
808 TC	0	273	1700	1145	357
813 TC	0	1166	2100	10000	6800
828 TC	0	189	536	903	693
830 TC	0	693	1300	525	336
838 TC	0	483	989	977	914
722 OC	63	326	21	1523	2090
754 OC	0	0	11	11	336
764 OC	0	21	83	861	578
800 OC	0	25	32	21	32
841 OC	0	74	95	200	326
842 OC	0	42	154	431	126
852 OC	0	179	35	536	599
854 OC	42	1271	105	28	126

APPENDIX 4.2

The body weights (kg) of each animal at intervals during the experiment (Series II)

ANIMAL NO	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 7
708 C	42.5	44.0	40.0	43.0	40.0	37.5
751 C	43.0	44.0	44.0	44.0	44.0	43.0
757 C	48.0	49.5	47.5	46.5	46.0	47.5
762 C	44.5	46.5	47.0	46.5	46.5	45.5
763 C	46.0	44.5	43.5	43.0	44.0	42.5
774 C	40.5	42.0	41.5	40.0	39.5	38.5
777 C	42.5	44.5	43.0	44.0	43.0	41.0
759 TC	44.5	45.0	44.0	44.5	44.0	43.5
765 TC	47.0	47.5	46.5	47.5	46.0	45.0
775 TC	44.5	45.0	45.0	43.5	42.0	41.5
808 TC	39.0	39.5	41.0	41.0	42.0	38.5
813 TC	44.5	43.5	43.5	43.5	41.0	36.5
828 TC	47.0	48.0	48.0	48.5	48.0	48.0
830 TC	38.5	39.5	38.5	39.0	37.5	36.0
838 TC	37.0	37.5	37.0	38.0	37.5	35.5
722 OC	39.5	41.5	42.0	42.0	41.5	37.5
754 OC	42.5	47.0	47.0	45.5	46.0	47.0
764 OC	44.5	44.5	43.0	42.5	42.0	40.0
800 OC	39.5	40.5	39.5	40.5	39.5	39.0
841 OC	40.0	40.5	41.0	40.0	36.5	33.0
842 OC	41.0	43.0	41.5	42.0	41.5	37.5
852 OC	47.5	38.0	38.5	39.0	37.5	35.0
854 OC	38.5	39.0	39.5	41.0	41.0	39.0

APPENDIX 4.3 The mean daily food intakes (g/d) for each animal (Series II)

ANIMAL NO	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7
708 C	915	822	904	619	666	299	178
751 C	1005	1068	989	1000	1000	914	700
757 C	1102	1200	1029	1000	1000	914	700
762 C	1076	1200	1029	1000	1000	914	700
763 C	863	1200	1029	1000	1000	914	700
774 C	951	1036	684	1006	549	700	694
777 C	994	973	1029	1164	684	700	673
759 TC	1200	1121	1200	1171	869	915	730
765 TC	1200	1200	1189	1154	882	670	309
775 TC	1200	1128	456	487	420	538	161
808 TC	1169	1142	1156	987	837	646	445
813 TC	1091	705	779	691	214	186	99
828 TC	1200	1165	1200	1171	915	873	908
830 TC	1129	1051	821	791	797	669	277
838 TC	868	1016	704	1065	1000	667	454
722 OC	1193	1200	1029	926	798	665	241
754 OC	1200	1200	993	1000	1000	1000	671
764 OC	1151	603	588	919	329	361	356
800 OC	1200	1131	1001	969	961	811	709
841 OC	1200	1159	951	444	241	225	220
842 OC	1200	1179	1055	902	768	491	691
852 OC	1187	1200	1123	864	682	700	582
854 OC	1200	1200	1181	1171	957	513	591

APPENDIX 4.4

Plasma Ca concentrations (mg/l) for each animal at intervals during the experiment (Series II)

ANIMAL NO	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 7
708 C	93.7	97.9	92.9	96.2	91.3	93.0
751 C	94.2	97.6	95.5	89.8	83.3	83.8
757 C	101.6	93.6	95.3	91.0	86.6	86.3
762 C	93.3	87.4	99.8	90.3	84.3	82.6
763 C	95.3	97.0	96.0	85.1	81.3	82.4
774 C	105.0	99.3	96.7	103.7	90.9	98.6
777 C	98.5	96.6	101.1	98.8	95.0	98.7
759 TC	98.2	94.8	95.4	94.3	106.3	81.6
765 TC	99.6	96.2	98.4	100.4	101.9	93.4
775 TC	98.1	99.7	95.9	96.5	98.3	94.3
808 TC	92.7	96.6	94.6	102.4	99.6	80.0
813 TC	101.2	100.5	99.3	104.4	95.1	87.2
828 TC	98.7	93.8	94.5	101.5	101.1	88.1
830 TC	94.2	91.5	96.7	97.4	101.7	89.2
838 TC	99.7	97.4	103.2	96.4	106.1	85.5
722 OC	99.1	90.3	91.6	84.6	76.1	78.2
754 OC	110.0	100.6	91.4	83.9	99.9	88.2
764 OC	102.9	97.9	95.5	92.3	89.8	87.2
800 OC	98.6	98.8	96.3	89.1	84.9	88.4
841 OC	93.0	91.2	89.8	78.3	77.9	65.7
842 OC	97.1	97.8	126.4	95.0	91.1	79.8
852 OC	94.1	89.7	90.9	85.8	86.1	79.0
854 OC	97.2	95.3	94.9	92.8	88.3	-

APPENDIX 4.4 Plasma P concentrations (mg/l) for each animal at intervals during the experiment (Series II)

ANIMAL NO	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 7
708 C	41.1	80.6	53.3	69.8	97.3	79.9
751 C	75.5	92.9	85.0	73.3	89.7	91.3
757 C	44.6	82.6	47.3	44.1	58.2	54.5
762 C	72.8	63.6	64.6	65.9	83.5	71.2
763 C	50.4	78.2	60.6	68.7	93.6	76.1
774 C	51.2	100.6	92.3	70.5	70.4	99.7
777 C	66.6	73.9	70.3	58.1	67.1	73.4
759 TC	66.2	49.4	54.1	40.8	27.6	46.4
765 TC	71.2	71.5	72.4	55.6	43.1	36.6
775 TC	76.6	67.0	51.8	58.1	48.6	49.5
808 TC	72.3	72.6	58.0	31.4	35.0	42.9
813 TC	55.1	44.7	44.9	26.7	39.6	67.0
828 TC	74.3	63.4	52.8	43.4	37.9	49.8
830 TC	74.0	53.8	23.8	27.9	22.5	30.9
838 TC	56.4	70.6	63.3	65.9	39.2	38.1
722 OC	61.0	76.4	66.4	57.9	75.5	61.1
754 OC	74.1	68.2	63.5	60.7	59.0	80.7
764 OC	67.1	76.3	65.8	71.3	92.1	77.0
800 OC	74.3	72.3	76.6	62.0	84.2	77.7
841 OC	83.6	71.9	72.9	73.4	106.8	98.4
842 OC	65.4	57.1	41.1	58.2	55.1	88.3
852 OC	75.2	65.1	68.2	73.4	92.3	96.2
854 OC	55.5	52.9	68.9	85.4	79.8	-

APPENDIX 4.5a

The flow rates along the digestive tract of the lambs which ate 700g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	RUMINAL FLOW RATES			ABOMASAL FLOW RATES			ILEAL FLOW RATES		
		TOTAL (kg/d)	LIQUID PHASE (l/d)	SOLID PHASE (g/d)	TOTAL (kg/d)	LIQUID PHASE (l/d)	SOLID PHASE (g/d)	TOTAL (kg/d)	LIQUID PHASE (l/d)	SOLID PHASE (g/d)
757 C	700	5.97	5.54	436	6.39	5.96	338	3.59	3.30	259
762 C	700	7.73	7.19	539	8.00	7.53	361	3.48	3.22	236
751 C	700	6.15	5.70	444	5.98	5.51	300	2.89	2.60	222
763 C	700	5.62	5.13	492	6.47	6.04	375	3.36	3.07	228
830 TC	700	6.01	5.58	435	6.21	5.87	257	4.43	4.16	198
808 TC	700	5.01	4.67	338	8.13	7.75	319	3.22	2.99	201
765 TC	700	4.51	4.14	372	4.69	4.41	210	2.85	2.65	168
852 OC	700	6.02	5.63	389	16.15	14.85	341	2.35	2.12	165

APPENDIX 4.5b The flow rates along the digestive tract of the lambs which ate 1000 g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	RUMINAL FLOW RATES			ABOMASAL FLOW RATES			ILEAL FLOW RATES		
		TOTAL (kg/d)	LIQUID PHASE (l/d)	SOLID PHASE (g/d)	TOTAL (kg/d)	LIQUID PHASE (l/d)	SOLID PHASE (g/d)	TOTAL (kg/d)	LIQUID PHASE (l/d)	SOLID PHASE (g/d)
757 C	1000	9.24	8.54	706	7.79	7.22	442	6.33	5.85	313
762 C	1000	10.69	10.07	618	9.22	8.69	490	4.32	3.95	318
751 C	1000	9.77	9.16	602	9.55	8.86	513	5.13	4.72	272
763 C	1000	6.27	5.78	493	9.26	8.74	523	4.14	3.88	324
828 TC	1000	7.58	7.19	393	11.41	10.88	428	6.08	5.66	281
759 TC	1000	6.22	5.68	539	8.99	8.62	362	4.57	4.23	252
838 TC	1000	7.88	7.31	570	9.56	9.07	395	5.37	4.97	287
754 OC	1000	11.02	10.36	660	18.00	17.10	472	5.57	5.30	275
800 OC	956	10.14	9.57	572	18.47	17.33	498	4.16	3.88	296
722 OC	895	8.42	7.89	533	19.36	19.06	476	6.23	5.68	289

APPENDIX 4.5c The flow of dry matter along the digestive tract of the lambs which ate 700g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	DM INTAKE (g/d)	RUMINAL FLOW OF DM			ABOMASAL FLOW OF DM			ILEAL FLOW OF DM		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	700	601	519	83	436	435	99	336	325	67	258
762 C	700	601	670	131	539	483	125	358	306	71	235
751 C	700	601	539	95	444	404	102	302	285	63	222
763 C	700	601	576	84	492	467	95	372	296	68	228
830 TC	700	603	530	95	435	354	98	256	279	82	197
808 TC	700	603	419	81	338	439	122	317	267	66	201
765 TC	700	603	442	70	372	284	75	209	227	60	167
852 OC	700	603	487	98	389	526	178	348	223	59	164

APPENDIX 4.5d The flow of dry matter along the digestive tract of the lambs which ate 1000g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	DM INTAKE (g/d)	RUMINAL FLOW OF DM			ABOMASAL FLOW OF DM			ILEAL FLOW OF DM		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	1000	862	858	152	706	571	131	440	419	106	313
762 C	1000	862	758	140	618	620	134	486	408	90	318
751 C	1000	862	745	143	602	638	123	515	371	98	273
763 C	1000	862	599	106	493	652	132	520	410	88	322
828 TC	1000	861	512	119	393	590	164	426	403	122	281
759 TC	1000	861	644	105	539	499	138	361	344	93	251
838 TC	1000	861	716	146	570	537	143	394	395	109	286
754 OC	1000	862	798	138	660	701	226	475	227	102	125
800 OC	956	824	727	155	572	694	204	490	379	84	295
722 OC	895	772	651	118	533	687	217	470	406	116	290

APPENDIX 4.5e

The flow of organic matter along the digestive tract of the lambs which ate 700g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	OM INTAKE (g/d)	ABOMASAL FLOW OF OM			ILEAL FLOW OF OM		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	700	540	364	57	307	262	45	217
762 C	700	540	398	69	329	248	50	198
751 C	700	540	326	60	266	236	47	190
763 C	700	540	395	56	339	244	48	196
830 TC	700	537	296	60	236	217	49	168
808 TC	700	537	361	70	291	201	50	151
765 TC	700	537	234	44	190	178	40	139
852 OC	700	537	385	73	312	181	40	141

APPENDIX 4.5f

The flow of organic matter along the digestive tract of the lambs which ate 1000g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	OM INTAKE (g/d)	ABOMASAL FLOW OF OM			ILEAL FLOW OF OM		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	1000	771	490	75	415	310	67	243
762 C	1000	771	527	63	464	331	60	271
751 C	1000	771	548	50	498	304	65	239
763 C	1000	771	574	60	514	335	61	274
828 TC	1000	767	476	89	387	315	83	232
759 TC	1000	767	404	72	333	268	55	213
838 TC	1000	767	441	75	366	313	64	249
754 OC	1000	771	539	92	447	155	65	90
800 OC	956	737	553	88	465	308	58	250
722 OC	895	690	543	78	465	329	77	252

APPENDIX 4.6a The flows of phosphorus along the digestive tract of the lambs which ate 700g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	P INTAKE (g/d)	RUMINAL FLOW OF P			ABOMASAL FLOW OF P			ILEAL FLOW OF P		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	700	3.92	8.94	6.03	2.91	10.36	8.46	1.90	4.75	0.64	4.12
762 C	700	3.92	11.92	8.62	3.30	12.68	10.84	1.84	3.70	0.41	3.29
751 C	700	3.92	9.89	7.48	2.41	10.21	8.81	1.40	1.41	0.39	1.03
763 C	700	3.92	8.32	5.94	2.38	9.57	7.49	2.07	1.98	0.41	1.57
830 TC	700	4.22	5.61	2.95	2.66	5.29	3.88	1.41	3.81	0.73	3.08
808 TC	700	4.22	5.56	3.33	2.23	6.83	5.05	1.79	5.89	0.74	5.15
765 TC	700	4.22	5.14	3.30	1.84	5.61	4.28	1.33	3.90	0.46	3.45
852 OC	700	4.22	8.25	6.09	2.16	9.82	7.51	2.31	2.77	0.44	2.34

APPENDIX 4.6b The flows of phosphorus along the digestive tract of the lambs which ate 1000g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	P INTAKE (g/d)	RUMINAL FLOW OF P			ABOMASAL FLOW OF P			ILEAL FLOW OF P		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	1000	5.71	12.89	8.25	4.64	11.25	9.31	1.94	5.18	0.85	4.33
762 C	1000	5.71	14.39	10.48	3.91	14.20	12.60	1.60	4.91	0.66	4.25
751 C	1000	5.71	12.09	9.78	2.31	13.76	12.14	1.62	2.41	0.65	1.76
763 C	1000	5.71	9.61	5.80	3.81	10.56	8.57	1.99	4.31	0.59	3.71
828 TC	1000	6.03	6.17	3.53	2.64	9.33	6.66	2.67	5.92	0.83	5.09
759 TC	1000	6.03	6.14	4.11	2.03	8.54	6.05	2.49	6.23	1.13	5.11
838 TC	1000	6.03	8.68	4.99	3.69	8.99	6.24	2.75	5.86	0.88	4.98
754 OC	1000	5.71	12.83	8.80	4.03	14.30	11.71	2.58	5.08	0.69	4.39
800 OC	956	5.46	13.00	10.59	2.41	12.39	9.82	2.57	4.26	0.55	4.42
722 OC	895	5.11	10.47	7.76	2.71	11.63	8.98	2.65	3.48	0.52	2.96

APPENDIX 4.7a The flows of calcium along the digestive tract of the lambs which ate 700g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	Ca INTAKE (g/d)	RUMINAL FLOW OF Ca			ABOMASAL FLOW OF Ca			ILEAL FLOW OF Ca		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	700	5.28	4.06	0.72	3.34	6.71	6.25	0.46	6.08	0.63	5.45
762 C	700	5.28	5.11	1.01	4.10	6.35	7.08	-ve	6.28	1.03	5.25
751 C	700	5.28	4.47	0.80	3.67	5.29	5.34	-ve	4.82	0.88	3.94
763 C	700	5.28	3.82	0.67	3.15	5.44	5.56	-ve	5.27	0.80	4.47
830 TC	700	6.01	3.94	1.06	2.88	4.66	4.41	0.25	5.48	0.79	4.69
808 TC	700	6.01	3.86	0.89	2.97	6.78	6.28	0.50	7.61	0.78	6.83
765 TC	700	6.01	3.30	0.75	2.55	4.39	3.97	0.42	5.20	0.66	4.54
852 OC	700	6.01	4.05	1.13	2.92	6.59	5.79	0.80	4.11	0.68	3.43

APPENDIX 4.7b The flows of calcium along the digestive tract of the lambs which ate 1000 g/day (Series II)

SHEEP NO	FEED INTAKE (g/d)	Ca INTAKE (g/d)	RUMINAL FLOW OF Ca			ABOMASAL FLOW OF Ca			ILEAL FLOW OF Ca		
			TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)	TOTAL (g/d)	LIQUID PHASE (g/d)	SOLID PHASE (g/d)
757 C	1000	8.04	6.89	1.45	5.44	8.02	7.43	0.59	7.01	1.11	5.90
762 C	1000	8.04	6.46	1.51	4.95	7.72	7.47	0.25	7.68	1.30	6.38
751 C	1000	8.04	5.40	1.37	4.03	8.54	7.44	1.10	6.63	1.32	5.31
763 C	1000	8.04	6.42	1.27	5.15	8.31	8.04	0.27	8.88	1.16	7.72
828 TC	1000	8.59	4.37	0.86	3.51	7.89	8.27	-ve	8.23	1.08	7.15
759 TC	1000	8.59	4.76	1.14	3.62	7.76	6.64	1.12	7.88	0.93	6.95
838 TC	1000	8.59	5.36	1.17	4.19	8.34	7.16	1.18	8.05	0.80	7.26
754 OC	1000	8.04	6.26	1.14	5.12	12.21	11.12	1.09	8.97	1.22	7.75
800 OC	956	7.68	6.08	1.63	4.45	8.89	9.18	-ve	7.25	0.70	6.55
722 OC	895	7.19	4.71	1.34	3.37	7.41	6.29	1.12	5.59	1.02	4.57